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EDITORS
WILLIAM ALBERT SETCHELL
RICHARD M. HOLMAN
THOMAS HARPER GOODSPEED



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INTERSPECIFIC HYBRIDIZATION IN NICOTIANA

I. ON THE RESULTS OF BACKCROSSING THE F_1 SYLVESTRIS-TABACUM HYBRIDS TO SYLVESTRIS

BY

THOMAS HARPER GOODSPEED AND ROY ELWOOD CLAUSEN

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I. ON THE RESULTS OF BACKCROSSING THE F₁ SYLVESTRIS-TABACUM HYBRIDS TO SYLVESTRIS

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INTRODUCTION

In previous reports (Clausen and Goodspeed, 1916, and Goodspeed and Clausen, 1917) we have discussed in a general way the results of our long-continued studies of inheritance in interspecific hybrids between *Nicotiana sylvestris* and varieties of *N. Tabacum*. The presentation of the more detailed data has unfortunately been very much delayed. In the one article in our program which has been published (1917) we have presented in detail, with numerous illustrations, our evidence for the correspondence of the F_1 *sylvestris-Tabacum* hybrid and its particular *Tabacum* parent. It is the purpose of the present article to describe and illustrate the results of backcrosses of the F_1 *sylvestris-Tabacum* hybrids with *sylvestris*. The results of backcrosses of these hybrids with their *Tabacum* parents have led us into a number of more or less distinct lines of investigation which we hope to take up shortly in future numbers of this series.

By way of introduction it seems well to go over briefly the ground covered in our previous papers. It was pointed out a number of years ago (Goodspeed, 1913) that the F_1 *sylvestris-Tabacum* hybrids are not completely sterile, as has often been supposed. Their pollen appears to be almost entirely functionless, although microscopic examination discloses the presence of a few grains, normal in appearance, scattered among the great numbers of abortive and functionless grains. Even those pollen grains which appear to be normal, however, have never been observed to germinate. Some of the ovules, on the other hand, are capable of fertilization and production of viable seed. It has never been found possible to obtain viable seed by self-pollination of hybrid plants, for self-pollinated flowers are always abscised, whether enclosed in paraffin bags or left unprotected, as may be safely done in an isolated greenhouse. It is possible, however, to secure retention of capsules by using pollen of either of the parent species, and capsules thus retained normally contain a few viable seeds. Curiously enough, success of pollination appears to be favored by castration of the flowers. Earlier observations by Goodspeed and Ayres seemed to indicate difficulty in getting controlled back pollinations in the field, although it was easy to secure retention of capsules in the greenhouse, especially when the plants were subjected to conditions of low nutrition. Later

observations do not indicate that conditions under which the plants are grown have very much effect upon capsule retention, for vigorous F_1 plants have been observed to retain every capsule when pollen from either *sylvestris* or *Tabacum* was applied to *castrated flowers not enclosed in paraffin bags*. Under field conditions a few capsules are retained by the F_1 hybrids, but the great majority of flowers are abscised, presumably from lack of successful pollination. Most of the capsules which are retained contain a few seeds, and these seeds must obviously have resulted from chance pollen transfer from *sylvestris* or the various *Tabacum* varieties and their derivatives. Taken altogether, the various F_1 *sylvestris-Tabacum* hybrids probably represent as close an approach to complete sterility as it is possible to get without precluding the possibility of obtaining some viable seed.

Six very distinct *Tabacum* varieties, representing extreme forms within the species, have been crossed with *sylvestris* on one or more occasions. The conclusions which have been reached from these studies may be briefly summarized as follows:

1. F_1 in each case is practically a replica on an enlarged scale of the particular *Tabacum* parent involved in the hybrid.

2. Backcrosses with *sylvestris* give two broad classes of individuals: aberrant forms, which are almost completely sterile and produce no seed on self-pollination, and forms closely approximating *sylvestris*, which are partially fertile and on self-pollination in successive generations lead rapidly to the establishment of fully fertile lines in all respects identical with *sylvestris*.

3. Backcrosses with the *Tabacum* parent give three general classes of individuals: aberrant, nearly completely sterile forms which give no progeny on self-pollination; *Tabacum*-like forms which are, also, nearly completely sterile and give no selfed progeny; and *Tabacum*-like forms which are partially fertile and by successive generations of selfing rapidly lead to the establishment of fully fertile lines identical in all respects with the original *Tabacum* parent.

These statements represent slight modifications, made in the light of further investigations, from earlier statements as to the experimental facts which have been observed. Our first observation that the F_1 hybrid is a complete replica of its particular *Tabacum* parent still appears to us to be correct. On this point we have presented our experimental evidence in detail (1917) along with adequate illustrations, and we have discussed the evidence presented by other investigators. We know of but one other study that has been published

which was not considered in that article, namely, the work of Malinowski on crosses of "*Nicotiana atropurpurea* \times *Nicotiana sylvestris*." His account has just come to our attention. The extended descriptions and discussion are in Polish, but fortunately for us an unusually full English summary of fourteen pages accompanies the article. His *sylvestris*, judged by his descriptions and illustrations, is identical with ours. It is indeed a curious fact, this constancy of *sylvestris* as a species, for no matter from what sources we have obtained it, it has always conformed to a single type. The species appears to be truly monotypic and distinctly set off from other members of the genus. Malinowski's *atropurpurea* is probably very closely related to our *purpurea* (U.C.B.G. 25/06). It is clearly a *Tabacum* variety and illustrations and description correspond in general to our *purpurea*. Malinowski, however, notes that *sylvestris* is taller at maturity than *atropurpurea* and comes into bloom earlier. His figures for the height of *sylvestris*, 140–200 cm., accord well with our experience, but our *purpurea* is taller than *sylvestris* and blooms somewhat earlier when planted at the same time. It is probable that Malinowski's *atropurpurea* is therefore somewhat different from our *purpurea*, but it is obviously a derivative of the general stock of ornamental *Tabacum* forms, variously designated *macrophylla purpurea*, *purpurea*, *atropurpurea*, *sanguinea*, etc. Malinowski does not go into details concerning F₁. His complete statement, which is evidently a full translation of the Polish text, reads as follows:

The plants of this generation are like those of *N. atropurpurea*, but their flowers are brighter and they have the tube a little narrower. But the position of the flowers (they did not hang down), the leaves and the general aspect of the plant was like the *N. atropurpurea* individuals.

His observations, therefore, are in accord with those which we have published.

Obviously the study of inheritance in an interspecific hybrid presents a problem in analysis of much greater difficulty than that involved in the study of an intervarietal hybrid. In another report (Setchell, Goodspeed, and Clausen) it has been demonstrated that even the study of intervarietal hybrids within the species *Tabacum* may present rather formidable difficulties. We can reasonably expect, a priori, that, if the differences between distinct varieties of *Tabacum* are so extensive, the differences between the varieties of *Tabacum* and so distinct a species as *sylvestris* will present a still higher order of

difficulty. Accordingly, so far as possible, some means must be devised for simplifying the problem.

In the case of a series of segregation products of an intervarietal *Tabacum* hybrid, the simplification may be accomplished by a process of continued self-fertilization, the effect of which is eventually to establish derivative lines representing various recombination products of the two original parents. Now, obviously, in such a case the differences between the two original parents represent the maximum amount of difference, speaking in terms of Mendelian factors, which can exist between any two of these derivative lines, and any derivative line not identical with one or the other of the parents will usually differ in fewer germinal elements from either of the parents than the parents did from each other. Also, turning our attention to the differences existing between any two derivative lines, the chances in any given case that these approach the maximum amount of difference would be slight; they would on the contrary usually exhibit a smaller amount of germinal difference; and in so far as this amount had been lessened, the problem of analyzing it would have been correspondingly simplified. In the establishment of constant derivative lines the study of successive self-fertilized generations would also in itself provide a means of erecting a picture of the original germinal differences, for the attainment of constancy in a derivative line is a repeated process of germinal simplification until a homozygous condition is finally obtained. The hints thus obtained could subsequently be confirmed, and should be, by an analysis of the differences among the final group of forms obtained, including the original parents. The method outlined is exceedingly slow and laborious, but it seems to us there is no alternative but to follow it until a better one is devised.

In our work with interspecific hybrids we had thought to employ a similar method of analysis with such modifications as were dictated by the peculiar necessities of the case. The sterility of F_1 made it impossible to obtain seed by self-fertilization, consequently we were forced to resort to backcrossing either to *Tabacum* or to *sylvestris* in order to get a few seeds for the continuation of the studies. In order not to complicate the problem further, we have been careful not to introduce into our lines any germinal elements not represented in the original F_1 hybrid. Thus the F_1 *sylvestris-macrophylla* hybrid was backcrossed to *sylvestris* and to *macrophylla* and to no other forms. Inasmuch as the first backcross in each instance produced some partially fertile plants, the lines were subsequently established and

continued by self-fertilization of these partially fertile individuals. A priori, one might expect to obtain by this procedure two groups of lines, one leaning more to the *sylvestris* side, which had been derived from the backcross to *sylvestris*, and the other leaning to the *Tabacum* side because of its derivation from the backcross with *macrophylla*. These derivative lines having then been self-fertilized until they were constant and homozygous could be used for tests of the nature of the differences between the species, *sylvestris* and *Tabacum*, and of the effect of recombinations of the germinal elements from each.

Although this a priori line of reasoning sounds plausible enough and is in accord with Mendelian doctrine, it has not been substantiated by experiment. In the case of both backcrosses, the result has apparently been a rapid automatic elimination of all recombination products, so that we finally obtain again the two parent forms in their original condition without any modification whatsoever. In this paper we present evidence in support of this statement for backcrosses of two F_1 hybrids, viz., *sylvestris-macrophylla* and *sylvestris-purpurea*, to *sylvestris*. We have facilities for growing only about three thousand plants each year in our garden, consequently we have been forced to limit ourselves to a few lines of investigation in order to have adequate numbers of plants in them. We have not as yet, therefore, been able to follow out the results of backcrossing other F_1 *sylvestris-Tabacum* hybrids to *sylvestris*, but we hope to, later.

SYLVESTRIS-PURPUREA SERIES

In the prosecution of the *syvestris purpurea* studies the various hybrids and derivative lines have been given numbers as follows:

H34 = *purpurea* (U.C.B.G. 25/06) ♀ × *syvestris* (U.C.B.G. 69/07) ♂

H121 = F₁H34 ♀ × *syvestris* ♂

F₁H34 is the reciprocal of the F₁H33 described in our earlier paper (1917). In 1911 Professor Setchell made the backcross of pollen of *syvestris* on F₁H34. The seed obtained was germinated in 1913 and eighteen plants were grown in the field that year under the number F₁H121. Setchell has presented a description and illustration of *syvestris*, but, as far as plant characters are concerned, reference may be made to figure 1, where the plants of 14F₁H121P4 may be considered equivalent to *syvestris* at a late stage of development. A typical flower of *syvestris* is shown in figure 3c.

Among the eighteen plants of F₁H121, there was a remarkable range of variation in all characters. Figures 1 to 5 herewith tell the story better than any amount of description. In general appearance the extremes might be represented by plant 18 (fig. 2) on the one hand and by plant 4 (fig. 1) on the other. In the former we have a dwarf plant with thick, stiff, fleshy leaves and much branched main axis. Until the end of the normal growing season the flower buds remained undeveloped and even at the end of the season no flowers ever opened or matured beyond the stage shown in the largest flower on the plant as photographed. A number of the short lateral branches bore terminal masses of minute flower buds which were colorless throughout the season and never developed further. Plant 4, on the other hand, was a perfectly normal plant in every way; tall, robust, fertile, and was further an almost exact replica of *syvestris*. There were one or two other highly abnormal individuals in this population, but none so unusual in appearance as P18. Some of the flower types produced by plants in this population are shown in figures 3 and 4. A number of the plants bore flowers all of which showed either a three- or four-lobed stigma, and this condition was usually associated with the production of large numbers of three- and four-lobed corollas (see fig. 4). In a number of cases the anthers never opened and shriveled

early in the life of the flower, while in other cases the stigma was so far exserted and was receptive such a long time before the pollen in the same flower was shed that self-fertilization would have been impossible even had any normally matured ovules been formed. In



Fig. 1. At the left 13F₁H121P₄, a *syvestris-purpurea* backcross segregant closely resembling *syvestris*. At the right, a portion of a row of its selfed progeny, 14F₁H121P₄, exhibiting striking uniformity and agreement with pure

this latter connection it might be noted that all the flowers on one plant showed split and twisted corollas and pistils in which the placentae were foliaceous and no even rudimentary ovules were formed.

The expression of leaf, habit, and floral characters throughout was strongly suggestive of *syvestris*. Thus in flower color all but five of the eighteen plants were white-flowering and in only two cases was the

color more than a blush of pink or salmon in the throat of the corolla. The shape of the flower, in all but a relatively few cases, corresponded to that of *syvestris* or showed strikingly the influences of this parent, especially in the calyx characters, but also in the length and slenderness of the corolla tube. The photographs of entire plants and of their flowers emphasize these points.



Fig 2 Aberrant sterile segregants among the backcross progeny of the *syvestris purpurea* series

Four plants, P1, P4, P5, and P6, stood out rather sharply from the rest of the population by reason of their striking resemblance to *syvestris*. They were, also, the only individuals which were freely fertile, and seed under bag was formed by these plants alone. Undoubtedly a number of the remaining fourteen plants matured at least a proportion of normal ovules corresponding to that formed by the F_1 hybrid from which they were derived, but it was not possible at the time to examine this situation by pollination with parental or other normal pollens.

The progenies of three of the four plants noted above as corresponding most closely in appearance with *syvestris* were grown in F_2 .

those of P4, P5, and P6. These F_2 populations were strikingly uniform throughout, and equivalent in all respects to one another and to populations of *sylvestris*. Portions of the rows of F_2 H121P4 and F_2 II121P6 are shown in figure 1 and in figure 5, respectively. About 150 plants were grown in these three F_2 progenies.

The photographs of the F_2 populations make very clear the fact that not only were the characters of the particular F_1 parents preserved in the F_2 progenies, but also that there was a strong tendency to emphasize the expression of the *sylvestris* characters so strikingly manifested in the F_1 parents. One example will suffice to indicate the similarity among themselves of the individuals making up all of the three F_2 populations. No characteristic of *sylvestris* is more striking under our conditions than the slow rate of growth of the young plant and the long period during which the rosette condition is maintained. Under ordinary field conditions the *Tabacum* varieties are past their first blooming period before the main axis of the *sylvestris* plants reaches its full height. If field conditions are not very uniform along a given row where *sylvestris* plants are growing, there may be a decided difference in the dates on which the various plants in the row open their first flowers. Now, of sixty-seven plants of $14F_2$ II121P4, thirty-six began to flower within a period of two weeks, and within three weeks practically all the plants were in bloom. This is a degree of uniformity in time of flowering not surpassed under ordinary conditions by *sylvestris* and much greater than that exhibited by F_1 H121. This uniformity within one of these F_2 populations in the case of a more or less physiological characteristic is mentioned here because it emphasizes the corresponding and even more striking uniformity in the expression of structural characters in these hybrids to be seen in the photographs. All plants were completely fertile so far as could be ascertained.

One F_2 selection of H121 was grown in F_3 . Fifty plants represented this pedigree. This population was, again, strikingly uniform and approximated *sylvestris* very closely in general appearance and individual character.

Of a total of eighteen plants in the original backcross progeny of the F_1 *sylvestris*-*purpurea* hybrid with *sylvestris*, then, four plants closely approximated *sylvestris* in every respect and only these plants set seed on self-fertilization. Further progenies of three of them were grown, and these progenies were apparently to all intents and purposes pure *sylvestris* derivatives.



Fig. 3. Flower types each representing a single plant of the backcross progeny of the *sylvestris-purpurea* series as compared with that of *sylvestris* (e). *a* and *c* are from fertile plants closely resembling *sylvestris*. *b* and *d* are from sterile plants.



Fig. 4. Additional flower types each representing a single plant of the backcross progeny of the *sylvestris-purpurea* series. These flowers were produced by sterile, aberrant plants.



Fig. 5. At the left, 13F₁H121P6, a *sylvestris-purpurea* backcross segregant closely approximating *sylvestris*. At the right, a portion of a row of its selfed progeny exhibiting close agreement with *sylvestris*.

SYLVESTRIS-MACROPHYLLA SERIES

The numbers and their several significancies pertinent to the *sylvestris-macrophylla* series are as follows:

H38 = *macrophylla* (U.C.B.G. 22/07) ♀ × *sylvestris* (U.C.B.G. 69/07) ♂.

H154 = later hybrid with same parentage as H38.

H124 = F₁H38 ♀ × *sylvestris* ♂.

H210 = F₁H154 ♀ × *sylvestris* ♂.

F₁H38 has been fully described and figured in our previous report (1917; plate 39, fig. 1, plate 44, fig. 1, and plate 47). The backcross was made in the greenhouse in February, 1913. between plants which had been transplanted to pots in the fall of 1912. One of a number of cross-pollinations was successful, the capsule containing twenty-nine well-formed seeds. Twenty-five seeds germinated and of the twenty-five seedlings, seventeen were brought to maturity in the field during the summer of 1913.

Plants of F₁H124 are illustrated in figures 6 and 7 and individual flowers are shown in figures 8 and 9. This hybrid exhibited very much the same series of unusual or abnormal types as did F₁H121 described above. Thus a number of plants showed split and bent corollas and in one case a majority of the flowers were six-lobed as to corolla limb and every flower bore six stamens. A particularly abnormal type which succeeded in producing only a relatively few mature flowers is shown in figure 7a. The main axis in this case bore at its apex a few fully open flowers while all the buds on the short, weak laterals and most of the buds on the terminal inflorescence abscissed early.

F₁H124 differed in the aggregate from F₁H121 in two important respects, although the differences were possibly due to the small number of plants grown in the two cases. In the first place but one plant (P15, cf. fig. 6) showed a very decided, if not almost exact, resemblance to *sylvestris*. It will be remembered in this connection that a number of plants of F₁H121 were of this type. The generally diminutive size of practically all of the plants of F₁H124 gave emphasis to this lack of similarity to *sylvestris*. Thus there were a few plants under two feet in height which in leaf characters and to some extent in flower characters resembled *sylvestris* closely, while the

dwarfed, much branched habit produced a general appearance strikingly different from that of this species. In the second place only P15 was freely fertile, at least it was the only plant which made seed



Fig. 6. At the left, 13F, H124 P15, a *sylvestris-macrophylla* backcross segregant closely resembling *sylvestris*. At the right, a portion of a row of its selfed progeny, exhibiting striking uniformity and agreement with *sylvestris*.

under bag. In one or two cases, notably P10 (cf. fig. 7b), a little open-pollinated seed was formed, but repeated artificial self-pollinations failed to give protected seed.

On the other hand, the white color and the general configuration of the flower and the general leaf characters peculiar to *sylvestris* were exhibited in practically all the plants of F_1 II124. Five of the seventeen plants in this population bore colored flowers, the color again varying in intensity from the shade of red characteristic of F_1 H38 to a very delicate pink.



Fig. 7. Aberrant, sterile plants among the backcross progeny of the *sylvestris-macrophylla* series.

A population of twenty-five plants was grown in F_1 from seed of F_1 II124P15. A small portion of the row is shown in figure 6. As in the case of F_1 progenies in the H121 series, F_1 H124 exhibited a remarkably close similarity to *sylvestris*. The fact that F_1 II121 showed a relatively restricted range of variation in time of flowering was taken as evidence of similarity within the populations. In the case of F_2 II124 the range of dates for first flowers is even more restricted in that seventeen plants bloomed within a week and the entire population within twelve days. The illustration of F_2 plants shows their striking resemblance to *sylvestris* in general appearance and habit. All F_2 plants were freely self-fertile.

Three plants of F_2 H124 were grown in F_3 and gave populations identical within themselves and indistinguishable one from the other and from the parent plants.

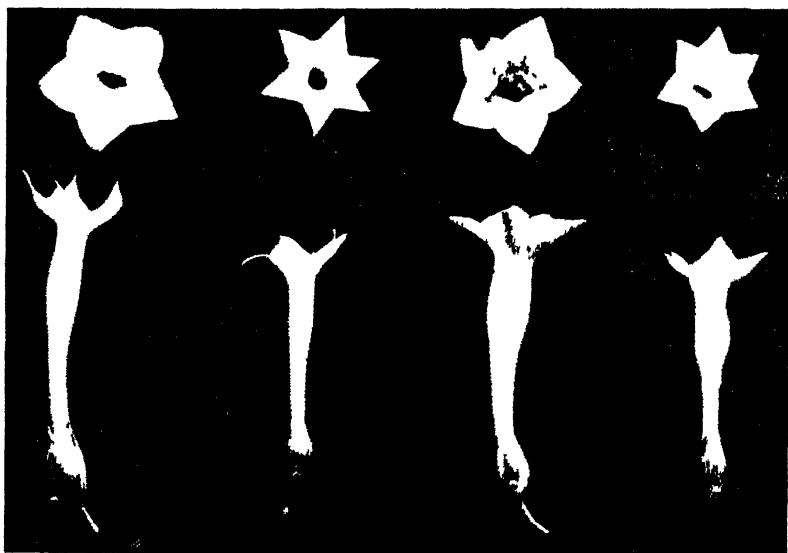


Fig. 8. Flower types each representing a single sterile plant of the backcross progeny of the *sylvestris-macrophylla* series.



Fig. 9. Additional flower types each representing a single plant of the backcross of the *sylvestris-macrophylla* series. The flower at *a* was from 13F,H124P15, the only fertile plant in the population and the only one which showed close resemblance to *sylvestris*.

F₁H210, which was grown in 1916, is the equivalent of F₁H124 and exhibited the same situation as described above for this latter hybrid. In figure 10 two plants of F₁H210 are shown. Though immature, P17 resembles *sylvestris* and it proved fertile. P1 is characteristic of the individuals which made up the bulk of the population. They showed no marked resemblance to the parental or to any other species, and all exhibited some peculiarity. This plant bore very delicate, white flowers which were early withering, and it was sterile.

NATURE OF SYLVESTRIS DERIVATIVES

In the foregoing part of this paper we have shown that the derivatives lines obtained from backcrossing the F_1 *sylvestris*-*Tabacum* hybrid to *sylvestris* are rapidly reduced to a condition of full equivalence to *sylvestris*. The question as to whether or not they contain any recombination elements whatever is, however, a difficult one to answer with assurance. If they do contain recombination elements, these elements appear to have no obvious morphological or physiological effect. The recombination elements derived from the *Tabacum* parent, if there are any, must then either be identical with those elements of the normal *sylvestris* genic system which they displace, or must be so nearly identical with them as to lead to no derangement in the functioning of the system and to no change in the morphological and physiological characteristics of the resultant *sylvestris* derivatives. Pending positive evidence to the contrary, however, we incline to the belief that the complete equivalence of these *sylvestris* derivatives to normal *sylvestris* is evidence of genetic identity with *sylvestris*.

As further evidence of the identity of these *sylvestris* derivatives with normal *sylvestris*, we have the results of crosses between the two forms. F_2 *sylvestris* derivatives from the *sylvestris*-*purpurea* series crossed with normal *sylvestris* gave F_1 and F_2 populations identical with normal *sylvestris* and fully fertile. This test, however, still does not establish absolute identity between the two forms.

Further tests have been made by crossing *sylvestris* derivatives with the original *Tabacum* parents. Thus in 1916 we grew twenty-five plants of F_1 H193, which was a hybrid of *purpurea* ♀ × 15F₁ H121P4-P36P100 ♂, the male parent an apparently constant *sylvestris* derivative from the *sylvestris*-*purpurea* series. The *sylvestris* derivative represented the results of two generations of self-fertilization of the plant shown in figure 1, and no differences could be detected between it and normal *sylvestris*. For comparison with it we had in an adjacent row fifty plants of F_1 H212, an F_1 *sylvestris*-*purpurea* hybrid. The two populations, 16F₁ H193 and 16F₁ H212, were identical in all respects; in growth characters, sterility, vigor, floral characters, and in all obvious morphological characters, except for the fact that 16F₁ H193 was distinctly dimorphic as to leaf-base type.

The details are shown clearly in figures 11 and 12. Figure 11*b* illustrates the leaf type characteristic of *purpurea*, the deeply constricted leaf base being its distinctive feature. Figure 11*a* illustrates the leaf-base type characteristic of the F_1 *sylvestris-purpurea* hybrid. It does not possess the marked constriction characteristic of *purpurea*. In figure 12 are reproduced photographs of two leaves representing the two leaf-base types of F_1 H193. It will be noted that the constricted



Fig. 10. Two plants of 16 F_1 H210, a backcross progeny of the *sylvestris-macrophylla* series. P1 was a sterile, aberrant form representative of the bulk of the population, while P17, although immature, resembled *sylvestris* and proved to be partially fertile.

form exactly reproduces the leaf-base type characteristic of *purpurea*, while the other form corresponds exactly to the leaf-base type of the normal F_1 *sylvestris-purpurea* hybrid. F_1 H193 separated sharply into these two types without any intergrading whatsoever, so far as we could determine. One plant in this population was an obvious stray, as shown by its complete fertility and lack of agreement in other respects with the remainder of the population. A census of the remaining twenty-four plants in the population gave eleven constricted and thirteen broad leaf base. This segregation was so striking and so unexpected that an additional sowing of the same seed was made in 1919. Among fifty plants of 19 F_1 H193, twenty-six had constricted and

twenty-four broad leaf base. From the two sowings we obtained 37 constricted : 37 broad, an exact agreement with a 1:1 ratio. We were unable to detect in either of these sowings any differences between the two types of plants, except that shown by the leaf base. Measurements of length and spread of corolla presented in table 1 exhibit no certain differences between them. The means given by these data are as follows:

	Mean spread	Mean length
Broad	42.42 \pm 0.24	55.58 \pm 0.23
Constricted	41.65 \pm 0.22	55.81 \pm 0.20
Difference	0.77 \pm 0.33	0.23 \pm 0.30

Now, it seemed self-evident that the explanation of the segregation in F_1H193 must be sought in the *sylvestris* derivative concerned in its parentage. Seed of $15F_1H121P4P36P100$, the immediate parent of this hybrid was not available, but additional selfed seed of its parent had been saved. Accordingly in 1919 we grew a population of forty-seven plants of $19F_3H121P4P36$, equivalent to the population which had produced the parent of F_1H193 . These plants unfortunately were not started early enough in the season, so that they did not bloom in time to make a proper study of them, nor was it possible to obtain further hybrid seed from them. As far as our observations extended, however, the population consisted entirely of typical *sylvestris* plants, and there was no segregation for the constricted type of leaf base. It is therefore necessary to explain how this particular *sylvestris* derivative was heterozygous for the factor *c* for constricted leaf base, and yet the population in which it occurred showed no segregants of that leaf-base type.

TABLE 1

DISTRIBUTION OF LENGTH AND SPREAD OF COROLLA IN $19F_1H193$.

One flower measured on each plant.

Leaf base	Spread in millimeters								Length in millimeters							
	39	40	41	42	43	44	45	46	53	54	55	56	57	58	59	
Broad.....	4	6	2	3	7	1	1	3	4	5	5	2	2	
Constricted.....	2	5	7	4	3	4	1	1	4	6	8	4	1	2	
Total.....	2	9	13	6	6	11	2	1	4	8	11	13	6	3	2	

In seeking for an explanation of the ultimate purification of *sylvestris* derivatives we are concerned more particularly, it seems to us, with a process of zygotic than with one of gametic elimination. When we consider a fertile backcross segregant closely approximating normal *sylvestris*, we can hardly consider such a segregant as anything but the result of a gamete of the *sylvestris* end of the recombination



Fig. 11. At the right a typical leaf of *purpurea*. At the left a typical leaf of the F_1 *sylvestris-purpurea* hybrid.

series in our F_1 hybrid fertilized by a normal *sylvestris* pollen grain. It is hardly conceivable that the F_1 gamete contained a genic system made up wholly of *sylvestris* elements. The nature of the backcross *sylvestris* segregants and their behavior indicates that they are not absolutely equivalent to normal *sylvestris*, and any lack of equivalence is doubtless due to the substitution of some *Tabacum* for *sylvestris* elements. The gamete, however, survived in spite of this fact, and was fertilized by a normal *sylvestris* pollen grain. As a consequence we should expect all gametes formed by such a plant to be capable potentially of surviving, for none of the gametes of such an individual

can contain a greater proportion of *Tabacum* elements than the one which entered into it, and the vast majority, if normal recombination takes place, would have a smaller proportion of such elements. Survival of gametes, however, is not a clear-cut phenomenon entirely referable to the genetic constitution of the gametes, but obviously depends in part on physiological conditions existing at the time of



Fig. 12. Typical leaf types of F_1H193 , an F_1 hybrid of a *sylvestris* derivative with *purpurea*. At the right, the constricted leaf base type corresponding to that of *purpurea*. At the left, the broad leaf base type corresponding to that of the normal F_1 *sylvestris-purpurea* hybrid.

production and functioning of the gametes. Accordingly, in looking at this problem of reduction of *sylvestris* derivatives to a condition of genetic identity with normal *sylvestris*, we must look at gamete elimination subsequent to the backcross as an interplay of intrinsic genetic and extrinsic physiological causes, with no class of gametes eliminated outright by reason of its genetic constitution, but with various percentages of certain classes of gametes, eliminated by reason of a greater sensitiveness to any unfavorable extrinsic conditions which may surround them at the time of their formation and functioning.

It is for this reason that we feel inclined to emphasize the importance of zygotic elimination in the absolute purging of these derivative *sylvestris* lines of all foreign *Tabacum* elements.

The operation of zygotic elimination may be pictured as follows. For simplicity of presentation, consider an F_1 gamete which contains a single section of *Tabacum* elements displacing a corresponding section of *sylvestris* elements, the rest of the genic system being made up entirely of *sylvestris* elements. It will be convenient to designate this section $(A \text{ ---- } X)_T$, and the homologous *sylvestris* section, $(A \text{ ---- } X)_s$. In the backcross we have formed a zygote of composition, $\frac{(A \text{ ---- } X)_T}{(A \text{ ---- } X)_s}$, the rest of the genic system being entirely *sylvestris*. Such an individual might be a typical *sylvestris* plant in appearance, if the *Tabacum* elements in such a setting were unable to perform the functions normal to them in a *Tabacum* system. Neglecting the possibility of crossing over, of which we know nothing in such cases, a plant of this constitution would give the following classes and ratio:

$$1 \quad \frac{(A \text{ --- } X)_s}{(A \text{ --- } X)_s} \quad : \quad 2 \quad \frac{(A \text{ --- } X)_s}{(A \text{ --- } X)_T} \quad : \quad 1 \quad \frac{(A \text{ --- } X)_T}{(A \text{ --- } X)_T}$$

The first class consists of normal *sylvestris* individuals, which naturally will survive; the second class reproduces the parent constitution, and will also survive; but the third class constitutes the zygotic eliminants. We consider that this *Tabacum* section is relatively harmless so long as it is balanced by a homologous set of *sylvestris* elements; but when it becomes homozygous, it cannot perform the developmental functions of the *sylvestris* elements which it has displaced, and as a consequence individuals of this constitution never develop. We conceive, therefore, that these *Tabacum* sections included in the genic systems of *sylvestris* derivatives are comparable in their behavior to the deficiencies which have been studied in *Drosophila*; and if the process which we have outlined applies to every *Tabacum* section which has been originally included in these *sylvestris* derivatives, it is obvious that continued self-fertilization would very soon throw them all out and thus reduce the derivative lines to a constitution genetically identical with normal *sylvestris*.

To return to F_1H193 , the F_1 population which exhibited dimorphism with respect to leaf base. In order to explain this case it is only necessary to consider that the *sylvestris* derivative contained a single *purpurea* section which included the factor *c* for constricted

leaf base. Its genetic constitution with respect to the *purpurea* section might then be represented as $\frac{(A-c-X)_p + R_s}{(A-c-X)_s + R_s}$, designating by R_s the *sylvestris* residue. Such a plant crossed with *purpurea* would produce two types of individuals in equal numbers in F_1 , viz.,

$$\frac{(A-c-X)_p + R_s}{(A-c-X)_p + R_p} \quad \text{and} \quad \frac{(A-C-X)_s + R_s}{(A-c-X)_p + R_p},$$

designating by R_p the *purpurea* residue homologous with R_s . The second class of plants are normal F_1 *sylvestris-purpurea* hybrids and should therefore be identical with normal hybrids of this type. But plants of the first class do not contain an entire *sylvestris* system opposed to an entire *purpurea* system. Instead, they have one section of the *sylvestris* system, a whole chromosome or less, displaced by a homologous *purpurea* section containing the factor c . They are therefore homozygous for c and exhibit constricted leaves like those of *purpurea*. In this way the dimorphic F_1 population may be explained in conformance with our hypothesis of the effect and behavior of *Tabacum* elements retained in *sylvestris* derivatives.

TABLE 2

DISTRIBUTION OF LENGTH AND SPREAD OF COBOLLA IN 19F₁II186.
One flower measured on each plant.

Spread	Classes in mm.	30	31	32	33	34	35	36	37	39	39	40	Total
	Nos. of individuals.	1		2	3	7	13	12	30	24	13	4	99
Length	Classes in mm.	46	47	48	49	50	51	52	53	54	55	56	
	Nos. of individuals.	1			3	17	17	24	19	13	3	2	99

So much for the *sylvestris* derivatives of the *sylvestris-purpurea* series. A single test has also been made of a *sylvestris* derivative of the *sylvestris-macrophylla* series. A plant of one of these derivatives, 15F₃H124P15P3P3, was crossed with *macrophylla*, using the latter as the female parent, giving F_1 H186. Twenty-five plants of F_1 II186 were grown in 1916 and in all respects they seemed to be equivalent to normal F_1 *sylvestris-macrophylla* hybrids. In 1919 an additional sowing of 100 plants of this population was grown, and a careful study of them disclosed the presence of a few plants which exhibited slight and rather intangible differences from the normal type of these

hybrids. Notes were taken individually on height, and length and spread of corolla in this population. The height data exhibited no significant differences from those normally obtained from F_1 *sylvestris-macrophylla* hybrids, but the data on length and spread of corolla, contained in table 2, show that one plant gave flower measurements rather unusual for such a hybrid. Aside from this one plant, however, the distributions are typical of normal *sylvestris-macrophylla* hybrids. There is therefore no really dependable evidence of impurity in this *sylvestris* derivative.

It is not our purpose to discuss further the theoretical issues involved in these studies, for the data herewith presented do not enable us to push them any farther than we have in our previous papers, save perhaps as respects the nature of the *sylvestris* derivatives. Attention and acknowledgment should, however, be made of East's valuable analysis of the results of crossing *N. rustica* var. *humilis* with *N. paniculata*. East presents an interpretation of his results essentially identical with ours. He also points out that he had announced his conclusion prior to the publication of our papers (East, 1915). The hybrids which we have investigated and the results, however, differ in some important particulars from those of East, and these should be borne in mind in judging the similarity between them. The F_1 *paniculata-humilis* hybrids are more highly fertile than the *sylvestris-Tabacum* hybrids, and self-fertilized seed can be obtained from them. Moreover, stable recombination lines have been established from this hybrid, whereas we have reached the conclusion, tentatively, that it is impossible to establish stable recombination lines following hybridization of *sylvestris* and *Tabacum* varieties. The difference between these two cases may be interpreted merely as a difference in extent of elimination of recombinations. In *paniculata-rustica* crosses the greater part of them are eliminated, but some survive; whereas in the *sylvestris-Tabacum* crosses all recombinations except those identical with the original parents seem to be eliminated.

SUMMARY

1. Three *sylvestris* derivative lines, originating from backcrosses of F_1 *sylvestris-purpurea* hybrids to *sylvestris*, have been studied and shown to become identical with normal *sylvestris*.

2. One *sylvestris* derivative line from the *sylvestris-macrophylla* series has also been demonstrated to be identical with normal *sylvestris*.

3. A cross of a *sylvestris* derivative from the *sylvestris-purpurea* series with *purpurea* resulted in a dimorphic F_1 , which may be interpreted as evidence of the existence of a section of *Tabacum* elements in the *sylvestris* derivative. Since the *sylvestris* derivative was heterozygous for this section of *Tabacum* elements, this provides presumptive evidence of eventual purification of *sylvestris* derivatives by a process of zygotic elimination.

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INHERITANCE IN NICOTIANA TABACUM

VI. A MENDELIAN ANALYSIS OF CERTAIN FLOWER FORM, FLOWER AND FILAMENT COLOR, AND LEAF-BASE CHARACTERS

BY

MOHAMMED A. KELANEY

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VI. A MENDELIAN ANALYSIS OF CERTAIN FLOWER FORM, FLOWER AND FILAMENT COLOR, AND LEAF-BASE CHARACTERS

BY

MOHAMMED A. KELANEY

Nicotiana Tabacum, the source of the tobacco of commerce, includes a highly diverse assemblage of varieties and forms. A number of attempts have been made to determine the mode of origin of these varieties and to erect schemes of classification for them. Those of Comes (1905) and Anastasia (1906), although differing in details, were based upon the hypothesis that fundamentally the varieties had arisen by derivation from relatively few basic types, mostly by hybridization. The Howards (1910; 1913) and Setchell, Goodspeed, and Clausen (1922) have shown, however, that the morphological criteria which these authors employed are not always indicative of genetic affinities, for a number of similar morphological characters have been found to differ in genetic constitution.

Although numerous breeding investigations have been conducted with *Tabacum*, not many of them have been carried far enough to arrive at a definite factor analysis of the characters under consideration. For the polymorphism of *N. Tabacum* is largely expressed in quantitative features, such as habit of growth; size of plant; size, shape, and number of leaves; and these characters often differ complexly in a Mendelian sense and some of them vary notably in response to environmental conditions. When proper methods are employed, however, it is possible to subject some of them to an accurate Mendelian analysis.

The *Nicotiana* investigations at the University of California originally were designed to determine taxonomic relations among the numerous varieties and species within the genus. Later, a comprehensive series of investigations was inaugurated for the purpose of elucidating the phenomena exhibited in interspecific hybridization.

These studies have largely been conducted on hybrids of *N. sylvestris* with varieties of *N. Tabacum*. It was soon found that accurate determination of the behavior of the interspecific hybrid required an exact knowledge of the Mendelian basis of as many characters as possible in *N. Tabacum*. Accordingly, a series of studies has been conducted for the purpose of determining these relations and establishing analytic varieties of *N. Tabacum* of known genetic constitution. The investigations described in this paper represent a part of this program, and they are therefore reported under the same general title.

The methods employed in the present investigation in obtaining pure seed, performing cross-pollinations, cleaning and sowing the seed, and caring for the cultures in the field are in general the same as those which have been described by Goodspeed (1912, pp. 126-131). Extreme care must be taken at each step to avoid contamination in the cultures. In the field each plant was staked and numbered, and notes of characters were recorded individually on printed blanks. When necessary, cultures were examined several times during the season. In studies of leaf characters, records were made not only from examination in the field, but also from sample leaves cut and preserved as herbarium specimens. The numbering system was a simple notebook page system with a prefix to indicate the year in which the cultures were grown and plant numbers for individual plants. Thus 22,212 P₁₁ indicates culture number 212 of 1922 and P₁₁ is the number of the individual plant.

In certain instances advantage was taken of crossing F₁ or other heterozygous plants to *N. sylvestris* in order to determine their gametic ratios. This method is based upon the observation of Goodspeed and Clausen (1917b) that F₁ interspecific hybrids of *N. sylvestris* with varieties of *N. Tabacum* are enlarged replicas of the *Tabacum* parent. An F₁ *Tabacum* hybrid crossed with *N. sylvestris* should, according to this conception, give a progeny exhibiting a diversity of forms corresponding to the types of gametes produced by it. The method must, however, be used with discretion; for certain specific characters, as will be shown in what follows, give an intermediate expression in F₁. With these restrictions the method has proved valuable in analytical work, because it often permits determination of gametic ratios without first synthesizing the necessary multiple recessive types, as would be required for application of the same method within the species itself.

SPECIES AND VARIETIES

The work herein reported was started in the fall of 1922 at the University of California with material kindly furnished by Professor R. E. Clausen. It consisted of two F_1 progenies and nine pure parental types. The varieties and species of *Nicotiana* grown in the University of California Botanical Garden have been obtained from a variety of sources. Their purity with respect to the characters under investigation is known as all of them have been grown in the pure line and studied for several generations at the University of California Botanical Garden (U.C.B.G.) at Berkeley.

The principal and most striking characteristics under study which distinguish the different types are summarized in Table 1. Of the *Tabacum* varieties, *purpurea* (*macrophylla purpurea*) and *calycina* have previously been described by Setchell (1912). *Apetala* and Klebs-normal have been described by Klebs (1916) and the strains used in this investigation were derived from material obtained from him. *Sessilifolia*, *auriculata*, and enlarged *auriculata* are derivatives of the *angustifolia-macrophylla* series of investigations described by Setchell, Goodspeed, and Clausen (1922).

TABLE 1

PRINCIPAL CHARACTER DIFFERENCES OF VARIETIES AND SPECIES EMPLOYED IN THESE INVESTIGATIONS

Strain	Field Number	Flower Form	Flower Color	Filament Color	Leaf-base
<i>N. tabacum</i> varieties:					
<i>purpurea</i>	014	normal	carmine	pink	constricted
<i>apetala</i>	019	apetalous	pink	light-green	lanceolate
<i>calycina</i>	035	calycine	red	light-green	lanceolate
<i>sessilifolia</i>	047	normal	red	light-green	broad
<i>auriculata</i>	083	normal	red	light-green	constricted
enlarged- <i>auriculata</i>	083	normal	red	light-green	constricted
Klebs-normal	111	Klebs-normal	pink	light-green	lanceolate
<i>N. sylvestris</i>	050	normal	white	light-green	broad

FLOWER FORM

The flower forms under investigation have been designated as normal, calycine, and the Klebs' types: Klebs-normal, lacerate, and apetalous. These may be briefly characterized as follows:

Normal.—Although the normal-flowering varieties exhibit distinctive differences in details of flower form, the general shape of the corolla is infundibuliform, with throat somewhat inflated, limb patent and almost pentagonal. The calyx is normal in size and entirely green.

Calycine.—The calycine flower is of the hose-in-hose form. The corolla is usually split on one side, sometimes twice split, and more or less curved. The characteristic splitting of the corolla is seen even in young buds. The calyx is enlarged and more or less petaloid, and may or may not have irregularly distributed green stripes. Sometimes strips of petaloid tissue extend the entire length of the calyx.

Klebs-normal.—In the Klebs-normal type the corolla is normal in form, but the calyx has a tendency to be petaloid at the tips; and, as will be shown later, the type has a different genetic constitution from that of other normal forms. This is the form called *typica* by Klebs.

Lacerate.—The lacerate flower is phenotypically of the split hose-in-hose form similar in appearance to that of *calycina*. The calyx is enlarged and more or less petaloid as in the calycine type.

Apetalous.—In apetalous flowers the corolla is entirely lacking and the calyx has an elongated, pinkish-green, fleshy tube with 3 to 5 of its tips more or less petaloid. Apetalous plants produced very small quantities of seed; but with sufficient care selfed seed may be obtained from them and they may be used as female parents in crosses with other forms. A normal quantity of seed is produced in crosses with other forms when pollen from apetalous plants is used. The reason for the scanty seed production of this type has not been ascertained, but apetalous segregants from crosses with other types, constantly exhibit it.

RESULTS OF PREVIOUS STUDIES

Setchell, Goodspeed, and Clausen (1922) reported that *calycina*, which produces calycine flowers, crossed with *virginica*, a normal-flowering variety, gave normal F_1 and a ratio of 3 normal: 1 calycine in F_2 . They explained their results on the basis of a single genic difference, *C-c*, for normal vs. calycine.

Klebs' (1916) investigations dealt with the behavior of a *lacerata* plant discovered originally as a mutant form in one of his cultures. He found that *lacerata* selfed gave a progeny of 1 Klebs-normal (*typica*): 2 lacerate: 1 apetalous. Klebs-normal and apetalous plants bred true on self-fertilization, but the lacerate plants always segregated in a 1:2:1 ratio. The proportion of apetalous plants in F_2 progenies was always below expectation and Klebs obtained a fair ratio only from backcrosses of lacerate to apetalous. He ascribed the deficiency of apetalous plants to their comparatively low viability. He explained his results on a single factor difference, which is represented in this paper by *Ap-ap* for Klebs-normal vs. apetalous.

The experiments described in this paper were initiated particularly for the purpose of determining the relationship of the Klebs' flower forms to the normal and calycine types which had already been studied in the U.C.B.G. experiments. At the same time advantage was taken of other differences in flower color, filament color, and leaf-base type which occurred among the varieties employed in the investigations, both in order to obtain additional evidence concerning their genetic differences and the relation of their factors to those for flower form.

EXPERIMENTAL EVIDENCE

NORMAL-APETALOUS SERIES

Normal \times apetalous crosses are represented by hybridization of *apetala* with *auriculata*, *purpurea*, and *sessilifolia*. The F_1 hybrids were uniform throughout and flower form was normal and closely resembled that of the normal parent.

In F_2 some difficulty was encountered in distinguishing between extreme cases of petaloid normal and lacerate plants. Some of the petaloid normal plants bore split hose-in-hose and normal flowers in variable proportions. The number of split hose-in-hose flowers on these plants was, however, low in proportion to that of the normal flowers, and the splitting was not so extreme as that characteristic of lacerate and calycine flowers. Such plants were classified as normal and the classification was checked by growing selfed progeny of a number of them, which invariably consisted wholly of normal plants.

F_2 results are recorded in Table 2. The appearance of lacerate plants in F_2 progenies demonstrates the presence of the factor *Ap* in the normal parents; for *Ap ap*, as shown by Klebs' work, determines the production of the lacerate type of plant. The F_1 plants, however,

were of the normal type and not of the lacerate type, which demonstrates that the normal flower form of these varieties differs genetically from that of Klebs-normal. Assuming that the Klebs' types all differ from normal in an additional recessive gene necessary with *Ap ap* and *ap ap* for the production of lacerate and apetalous flowers, a dihybrid segregation is to be expected in F_2 . This gene has been given the symbol, c^a , since, as will be demonstrated below, it is allelomorphic to the *C-c* pair of genes for flower form previously discovered. F_1 is then $Cc^a Ap ap$, and F_2 reduces to the ratio of 13 normal: 2 lacerate: 1 apetalous, on account of the impossibility of distinguishing between normal and Klebs-normal. F_2 results, as shown in Table 2, are in substantial agreement with this interpretation.

TABLE 2

F_2 SEGREGATION FOR FLOWER FORM. $P_1 = \text{NORMAL} \times \text{APETALOUS}$; F_1 NORMAL
($C c^a Ap ap$) SELFED

Garden Number	Normal	Lacerate	Apetalous
23166	82	9	5
182	71	15	6
24267	75	10	12
271	75	17	5
Total	303	51	28
Calculated, 13:2:1	310.3	47.8	23.9

In order to establish the correctness of these conclusions, a number of analytical crosses were made and F_3 progenies were grown from the different types of F_2 segregants. The results are reported in tables 3 to 6.

TABLE 3

SEGREGATION FOR FLOWER FORM IN APETALOUS BACKCROSSES
 $P_1 = \text{NORMAL} \times \text{APETALOUS}$, F_1 NORMAL \times APETALOUS
($C c^a Ap ap \times c^a c^a ap ap$)

Garden Number	Normal	Lacerate	Apetalous
23164	55	27	13
186	52	19	16
24268	28	8	8
272	19	15	13
Total	154	69	50
Calculated, 2:1:1	136.5	68.25	68.25

When F_1 normal from normal \times apetalous is backcrossed to apetalous, according to the foregoing analysis, segregation in the ratio of 2 normal: 1 lacerate: 1 apetalous is to be expected. Results of this backcross, reported in Table 3, are in substantial agreement with expectation, aside from the usual deficiency in the apetalous class.

TABLE 4

SEGREGATION FOR FLOWER FORM. P_1 = NORMAL \times APETALOUS; F_1 NORMAL
(*C ca Ap ap*), BACKCROSSED TO KLEBS-NORMAL AND *sylvestris*

Parentage	Garden Number	Normal	Lacerate
F_1 normal \times Klebs-normal	23167	80	19
	181	58	22
	24270	34	12
	274	36	12
Total		208	65
Calculated, 3:1		204.75	68.25
F_1 normal \times <i>sylvestris</i>	23190	62	14
	191	84	15
	24269	39	9
	273	32	7
Total		217	45
Calculated, 3:1		196.5	65.5

TABLE 5

SEGREGATION FOR FLOWER FORM IN PROGENIES OF SELFED NORMAL SEGREGANTS

Garden Number	Parent Number	Normal	Lacerate	Apetalous
24291	23166 P_4	50	-----	-----
297	167 P_2	48	-----	-----
24292	23166 P_5	35	-----	12
Calculated, 3:1		35.25	-----	11.75
24296	23166 P_{13}	35	5	3
300	167 P_{37}	37	7	1
307	186 P_7	35	3	5
308	186 P_{10}	39	6	1
310	186 P_{30}	36	8	4
Total		182	29	14
Calculated, 13:2:1		182.8	28.1	14.1

TABLE 6

SEGREGATION FOR FLOWER FORM IN PROGENIES OF SELFED LACERATE SEGREGANTS
(*ca ca Ap ap*)

Garden Number	Parent Number	Normal	Lacerate	Apetalous
24283	23164P ₁	16	24	6
285	164P ₁₀	12	22	9
286	164P ₁₃	10	22	17
288	164P ₁₉	11	27	9
Total		49	95	41
Calculated, 1:2:1		46.25	92.5	46.25
24290	23166P ₁	20	18	9
294	166P ₂₈	9	27	8
295	166P ₃₁	8	21	10
Total		37	66	27
Calculated, 1:2:1		32.5	65	32.5
24298	23167P ₄	13	22	4
301	167P ₂₇	17	18	8
302	167P ₄₄	14	17	9
303	167P ₆₀	13	23	8
Total		57	80	29
Calculated, 1:2:1		41.5	83	41.5
24304	23186P ₂	9	29	7
306	186P ₉	10	21	17
309	186P ₁₇	21	20	7
Total		40	70	31
Calculated, 1:2:1		35.25	70.5	35.25
Grand Total		183	311	128
Calculated, 1:2:1		155.5	311	155.5

When the F_1 is backcrossed to Klebs-normal and *sylvestris*, segregation should occur in the ratio of 3 normal: 1 lacerate. The results recorded in Table 4 are again in agreement with expectation. Results from the *sylvestris* backcross are expected to conform to this ratio because apetalous-*sylvestris* crosses produce a lacerate F_1 . This is one of the instances in which an F_1 *sylvestris*-*Tabacum* hybrid exhibits intermediacy for a particular character contrast, normal flowers of *sylvestris* vs. apetalous of *Tabacum*, but in general the F_1 is an enlarged replica of the *Tabacum* parent as in other crosses. It should be noted that *sylvestris* behaves like Klebs-normal in these crosses, rather than like the normal of the other *Tabacum* varieties employed in the investigations.

As a further test of the correctness of this analysis progenies were grown in 1924 from selfed selections of different flower form in the previous hybrid progenies. The derivation of these segregants may be determined by reference to tables 3 and 4. Data obtained from the selfed segregants, tables 5 and 6, agree satisfactorily with the proposed formulation. Plants classified as normal were of the expected genotypes and either bred true to normal, or gave the ratio of 3 normal: 1 apetalous, or the ratio, 13 normal: 2 lacerate: 1 apetalous, while those classified as lacerate in all instances gave the expected ratio of 1 normal: 2 lacerate: 1 apetalous. Two apetalous selfed plants, as expected, bred true to type. As a further proof that the lacerate segregants were of the same genetic constitution as Klebs' lacerate, three of them were backcrossed to apetalous as well as selfed. The following results show that they are of the same genic constitution as lacerate plants; i.e., heterozygous for only one factor:

Garden Number	Lacerate	Apetalous
24287	27	21
289	29	24
299*	28	15
	—	—
Total, 84		60
Calculated, 1:1	72	72

* This is probably simply another case of an extreme deficiency of apetalous plants.

The reader will also note that all the lacerate selections included in Table 6 gave the expected 1:2:1 ratio, whether they were derived from a backcross progeny or an F_2 population, proving that they were all of the same genic constitution.

TABLE 7

SEGREGATION FOR FLOWER FORM. P_1 = NORMAL \times KLEBS-NORMAL; F_1 NORMAL
(*C ca Ap Ap*), SELFED AND CROSSED TO OTHER FORMS

Parentage	Garden Number	Normal	Lacerate
F_1 normal selfed	24251	88	-----
F_1 normal \times apetalous	24253	27	22
Calculated, 1:1		24.5	24.5
F_1 normal \times Klebs-normal	24254	48	----
F_1 normal \times <i>sylvestris</i>	24252	49	----

Crosses were also made between the normal varieties and Klebs-normal. Only one of them, however, viz., *purpurea* × Klebs-normal, was carried to F_2 as well as backcrossed to apetalous, Klebs-normal, and *sylvestris*; the other three crosses were dropped because of lack of ground space. Furthermore, the normal varieties, as proved, have the same genetic constitution for flower form. The F_1 hybrids in all four crosses were uniform and the flower form normal.

In the season of 1924 one F_2 family of the *purpurea*-Klebs-normal series was grown. The calyx of about one-fourth of the plants showed the petaloid tendency which is characteristic of Klebs-normal; but the flower form of all plants was normal in all other respects. When F_1 was backcrossed to normal, segregation occurred in a ratio of 1 normal: 1 lacerate as expected. Backcrosses to Klebs-normal and *sylvestris* yielded progenies consisting entirely of normal-flowering plants. These data are assembled in Table 7. Evidently Klebs-normal is $c^a c^a Ap Ap$.

APETALOUS-CALYCINE SERIES

Since calycine and lacerate flowers so closely resemble each other as to be indistinguishable in mixed cultures, it became a matter of some interest to determine their genetic relationship. Accordingly crosses were made in 1922 of Klebs-normal and of apetalous with calycine. F_1 progenies of both crosses were uniformly of the calycine type. In F_2 and other segregating progenies some difficulty in classification was experienced because some of the normal-flowering plants produced a small proportion of lacerate flowers. Such plants were included in the normal class. On the whole, however, the distinction between split hose-in-hose (lacerate and calycine) plants and normal was sharper than in the normal-apetalous series. It was impossible to distinguish any difference between lacerate and calycine plants.

TABLE 8

SEGREGATION FOR FLOWER FORM. P_1 = CALYCINE × KLEBS-NORMAL; F_1 CALYCINE ($C c^a Ap Ap$), SELFED AND CROSSED TO OTHER FORMS

Parentage	Garden Number	Normal	Calycine and/or Lacerate
F_1 calycine selfed	24255	20	81
Calculated, 1:3		25.25	75.75
F_1 calycine × apetalous	24257	---	40
F_1 calycine × Klebs-normal	24258	22	25
Calculated, 1:1		23.5	23.5
F_1 calycine × <i>sylvestris</i>	24256	24	22
Calculated, 1:1		23	23

TABLE 9

SEGREGATION FOR FLOWER FORM. $P_1 = \text{CALYCINE} \times \text{APETALOUS}$; F_1 CALYCINE
($C c^a Ap ap$), SELFED AND CROSSED TO OTHER FORMS

Parentage	Garden Number	Normal	Calycine and Lacerate	Apetalous
F_1 calycine selfed	24275	3	39	5
Calculated, 1:14:1		2.9	41.2	2.9
F_1 calycine \times apetalous	24276	---	35	13
Calculated, 3:1			36	12
F_1 calycine \times Klebs-normal	24277	11	38	---
Calculated, 1:3		12.25	36.75	---
F_1 calycine \times <i>sylvestris</i>	24278	8	23	---
Calculated, 1:3		7.75	23.25	---

The close approximation to a unifactorial segregation of 3 calycine: 1 Klebs-normal obtained when F_1 calycine from calycine \times Klebs-normal was selfed, indicates that these two types differ in a single gene. The demonstration by Setchell, Goodspeed, and Clausen (1922) that calycine is a simple recessive to normal, and the foregoing results, which show that normal and Klebs-normal differ in a single gene, indicate that normal must be $CC Ap Ap$; Klebs-normal, $c^a c^a Ap Ap$; and calycine $cc Ap Ap$. The factor c must, therefore, be allelomorphous to c^a and dominant to it. The results of backcrosses of F_1 to apetalous, Klebs-normal, and *sylvestris* are in agreement with this analysis as shown by the data recorded in Table 8.

If this analysis is correct F_1 calycine from calycine \times apetalous must be heterozygous for two factors, $c c^a Ap ap$. On selfing such plants the progeny should segregate in a ratio of 1 Klebs-normal: 14 lacerate and calycine: 1 apetalous. Only one such population was grown but it is in close agreement with expectation. The backcrosses of F_1 to apetalous, Klebs-normal, and *sylvestris*, also recorded in Table 9, are likewise in agreement with this analysis.

CONCLUSIONS

On the basis of these facts and those previously determined by Klebs (1919) and Setchell, Goodspeed, and Clausen (1922), the author suggests that the following factors are concerned in the relations among normal, calycine, lacerate, apetalous, and Klebs-normal flower types:

$C-c-c^a$: a series of triple alleomorphs for flower shape; C = normal, c = calycine, and c^a in conjunction with the factors, Ap and ap , gives the Klebs' types. Dominance is in the order indicated; C over c and c^a , and c over c^a .

$Ap-ap$: normal vs. apetalous flower form. Effective only in conjunction with c^a with which it gives the Klebs' flower types as shown below.

In terms of these factors the genetic formulae of the different flower shapes represented in the parental varieties are as follows:

$CC Ap Ap$ = normal of varieties, *purpurea*, *sessilifolia*, and *auriculata*

$c^a c^a Ap Ap$ = Klebs-normal

$c^a c^a ap ap$ = apetalous

$c^a c^a Ap ap$ = lacerate

$cc Ap Ap$ = calycine

This analysis explains how lacerate plants may be obtained from a cross between apetalous and normal. When normal is crossed with Klebs-normal only normal plants appear; and on backcrossing F_1 normal to apetalous, the result is 1 normal: 1 lacerate. The factor c for calycine determines the calycine character whenever present in a homozygous condition or heterozygous for c^a . Thus such genotypes as $cc^a Ap Ap$, $cc ap ap$, $cc^a ap ap$, etc., are phenotypically calycine. This is well illustrated by the data obtained from the apetalous-calycine crosses and backcrosses in Table 9, especially in the backcross to apetalous where a 3 calycine: 1 apetalous ratio was obtained, which proves that c is dominant over c^a in the genotype $cc^a ap ap$ and determines a calycine expression. If this were not the case, the ratios obtained would obviously have been entirely different.

On looking over the data on backcrosses to *sylvestris* recorded in tables 4, 7, 8, and 9, the reader will notice that *sylvestris* consistently behaves like Klebs-normal. As previously indicated, these results are in agreement with the observation that the F_1 apetalous-*sylvestris* hybrid exhibits the lacerate flower form.

FLOWER AND FILAMENT COLOR

The red flower color of the *Tabacum* varieties, *calycina*, *sessilifolia*, and *auriculata* lies between rose-red and pomegranate purple of Ridgway's color scale. The carmine flower color represented by *purpurea* is very close to red but is of deeper color. Flowers of the Klebs-normal types are pink. *N. sylvestris* has white flowers with a yellowish tinge.

As respects flower color, Allard (1919) found that carmine \times pink gave F_1 carmine and F_2 , 3 carmine: 1 pink. The backcrosses gave consistent data. In F_3 pink segregants bred true for pink, and carmine bred true for carmine or gave again 3 carmine: 1 pink. Setchell, Goodspeed, and Clausen (1922) and Clausen and Goodspeed (1921) have studied the interrelations between carmine, red, pink, and white flower colors. Red and pink gave F_1 pink and F_2 , 3 pink: 1 red. Red and white gave F_1 dark pink and F_2 , 9 pink: 4 red: 3 white. After performing several backcrosses and testing different segregants they obtained conclusive evidence for the following factorial formulae:

$WW RR PP$ = carmine

$WW RR pp$ = pink

$WW rr pp$ = red

$uw RR pp$ = white of the *Tabacum* varieties tested

While looking for a morphological difference by which it would be possible to distinguish between carmine and red, the author's attention was attracted to a difference in filament color. In *purpurea* the upper two-thirds of the filaments of the anthers are decidedly pink, but the filaments of the red, pink, and white types are greenish-white. Studies of segregation of filament color in hybrid progenies were therefore made in order to determine the Mendelian basis of the difference.

EXPERIMENTAL EVIDENCE

Results from the present study on flower color agree with those reported previously so that there is no need to publish the author's data on this character. The purpose for including it within these investigations was primarily to ascertain whether or not there were any linkage relations between the flower color factors and other factors under investigation, and the relation between flower and filament colors.

The white flower color of *sylvestris* proved to be completely recessive to the colored types of *N. Tabacum*. In a backcross of F_1 carmine

from carmine \times pink to *sylvestris*, 59 plants were carmine to 54 pink. When the F_1 pink from red \times pink was backcrossed to *sylvestris*, the result was 222 red: 207 pink or nearly a 1:1 ratio in either case. These results demonstrate that the pink of Klebs' types is identical genetically with that previously employed in flower color investigations.

Carmine with pink filaments crossed with pink with green filaments produced an F_1 which bore carmine flowers with pink filaments. F_1 selfed, as shown by the data recorded in the first section of Table 10, gave an F_2 segregating approximately in a ratio of 9 carmine with pink filaments: 3 carmine with green filaments: 4 pink with green filaments. F_1 backcrossed to pink with green filaments, as shown by the second section in the same table, gave a progeny in the ratio of 1 carmine with pink filaments: 1 carmine with green filaments: 2 pink with green filaments. These results indicate that the filament color is controlled by a complementary epistatic factor, G , which manifests itself only in the presence of the flower color factor, P . Hence when P is absent the filaments are light green. These results were confirmed by growing progenies of the various types of segregants. All selfed pink flowering segregants bred true for pink flower and light green filaments. Carmine segregants with pink filaments, as shown in Table 11, either bred true to type or gave 9 carmine with pink filaments: 3 carmine with green filaments: 4 pink with green filaments, or 3 carmine with pink filaments: 1 pink with green filaments. Two carmine selections with green filaments gave progenies segregating in the ratio of 3 carmine with green filaments: 1 pink with green filaments. The parentage of these segregants may be determined by referring to Table 10.

Similar cases of developmental relations between color in different parts of plants have been reported by a number of investigators. Bateson (1909) found that in *Primulas* certain deep red spots occur in some varieties on the petals just external to the yellow eye. These spots are never found unless the stigma is red. The spot is not formed if the stigma is green, although the factor for red stigma may be present. Also the stigma may be red but no spot is found. Marie Sachs Skolinska (1921) also found such interrelations in her study of flower colors in hybrids between *N. Langsdorffii* \times *N. Sanderae*. Flower color is dependent upon the distribution of different pigments, which are of course controlled by genic factors. Of these the factor, F , determines the violet color, but when the factor, C , is also present the flower color is violet red. The factor, F , can manifest itself inde-

pendently of the factor, *C*, while the latter cannot manifest itself without the presence of the former. This phenomenon of the change of color by the influence of a supplementary factor is also similar to that which Bateson and Punnet (1909) have observed in the progenies of sweet pea hybrids.

TABLE 10

SEGREGATION FOR FILAMENT COLOR. P_1 = CARMINE FLOWER AND PINK FILAMENT \times PINK FLOWER AND GREEN FILAMENT; F_1 CARMINE FLOWER AND PINK FILAMENT ($P p G g$), SELFED AND CROSSED TO PINK FLOWER AND GREEN FILAMENT

Parentage	Garden Number	Carmine flower color		Pink flower color
		Pink Filament	Green Filament	Green Filament
$F_1 P p G g$ selfed	23166	54	12	17
	24251	45	14	27
Total		99	26	44
Calculated, 9:3:4		95.1	31.7	42.2
$F_1 P p G g \times p p g g$	23164	21	22	38
	167	16	15	34
	24253	12	12	25
	254	8	8	31
Total		57	57	128
Calculated, 1:1:2		60.5	60.5	121

TABLE 11

SEGREGATION FOR FLOWER AND FILAMENT COLOR IN PROGENIES SECURED BY SELFING SEGREGANTS WITH CARMINE FLOWERS AND PINK FILAMENTS, AND CARMINE FLOWERS AND GREEN FILAMENTS

Garden Number	Parent Number	Carmine flower color		Pink flower color
		Pink Filament	Green Filament	Green Filament
24285	23164 P_{10}	25	9	8
288	164 P_{19}	27	10	11
291	166 P_1	27	9	9
294	166 P_{29}	29	7	8
297	167 P_1	31	6	11
300	167 P_{12}	24	7	11
303	167 P_{20}	30	5	7
Total		193	53	65
Calculated, 9:3:4		175.0	58.3	77.7
Genotype: $PpGg$				
24283	23164 P_1	-----	37	9
290	166 P_1	-----	36	11
Total		-----	73	20
Calculated, 3:1		-----	69.75	23.25
Genotype: $PPGg$				
24293	23166 P_{30}	48	-----	-----
295	166 P_{14}	44	-----	-----
Genotype: $PPGG$				

LEAF-BASE TYPES

The varieties employed in these investigations differ strikingly in leaf-base type. With respect to this feature it is possible to distinguish five main forms which are here designated: constricted, lanceolate, broad, petioled, and short-petioled. These may be described briefly as follows:

Constricted.—In this type the leaves are sharply constricted at the base, nearly if not quite to the midrib, as shown in figure 1. The varieties of this class vary as respects auricle development.

Lanceolate.—Lanceolate leaves are sessile and taper gradually toward both base and apex, as shown in figures 2 and 3. They, like constricted, vary in auricle development.

Broad.—Broad leaves are also sessile and typically taper gradually to broad, partially-clasping basal lobes, as shown in figure 4. They do not ordinarily differ sufficiently from lanceolate to permit accurate classification in mixed progenies.

Petioled.—Petioled leaves possess a distinct petiole, which is variable in length and in wing development. Figure 5 illustrates a long-petioled type entirely free from wing development.

Short-petioled.—Figure 6 illustrates the short but distinctly petioled type with well-developed auricles and a slight wing development.

RESULTS OF PREVIOUS STUDIES

Lodewijks (1911) emphasizes the intermediacy of F_1 of Peru (petioled) \times White Burley (broad), and the complexity of F_2 segregation. New leaf characters appeared in F_2 that were not present in either parent.

Hayes (1912) studied correlation and inheritance of various characters of different pure types of American tobacco. He also found complex segregation for leaf shape in F_2 , but he believes that in most cases the results can be explained by the presence of a large number of factors.

Setchell, Goodspeed, and Clausen (1921; 1922) found that *angustifolia* (petioled) \times *macrophylla* (broad) gave short-petioled F_1 and a complex series of forms ranging from extremely long-petioled to very broad sessile types in F_2 . It was possible, however, to distinguish certain forms as centers of distribution in F_2 and a more distinct distribution into classes in F_3 and subsequent generations. Constant

derivatives of various leaf-base types were established, and the genetic relations of the different types were studied by intercrossing these derivative lines and by crossing them with the original parents. They found that petioled selections either bred true or gave 3 petioled: 1 broad, or 3 petioled: 1 constricted. Two short-petioled selections bred true to type, one repeated the complex F_2 segregation in F_3 , and another gave 3 short-petioled: 1 broad. Broad selections either bred



Fig. 1

Fig. 1. Typical leaf of 22131P, illustrating the constricted leaf-base of *N. Tabacum* var. *purpurea*.

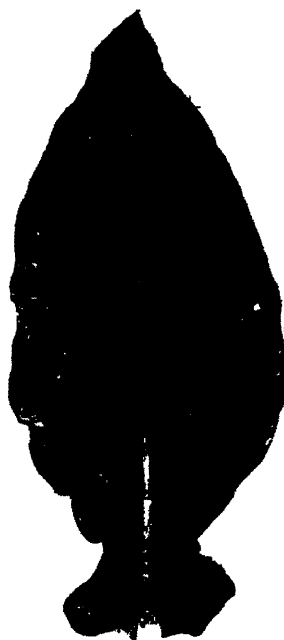


Fig. 2

Fig. 2. Typical leaf of 22113P, illustrating the lanceolate leaf-base type of *N. Tabacum* var. *apetalae*.

true to type or gave 3 broad: 1 constricted. The constricted selections bred true whenever tested. On the basis of these facts they offered the following formulation:

SS, petioled versus *ss*, broad.

SSLL, long-petioled versus *SSll*, short-petioled.

ssAA, broad versus *ssaa*, constricted.

The Howards (1913-14) studied a cross between two unnamed varieties classified as sessile broad. (One of these varieties, however,

as illustrated by their photographs, closely resembles a constricted and the other a lanceolate form. They state that F_1 had a larger indentation than either parent and that a variety of forms occurred in F_2 . Petioled forms bred true in F_3 , two cultures that had a small lamina in F_2 gave progenies of petioled and sessile forms, while three other similar cultures gave progenies in which a certain number of petioled forms occurred and a series of sessile forms, less than a fourth

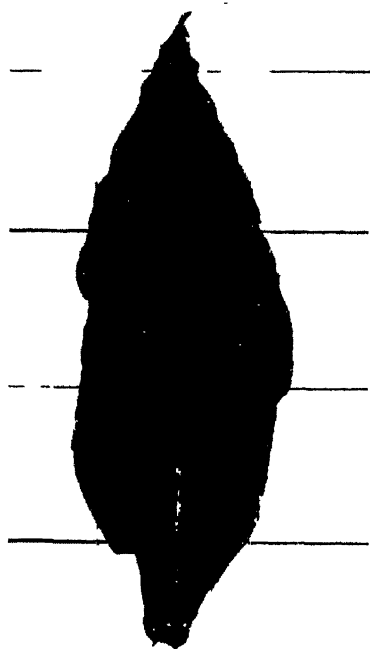


Fig. 3

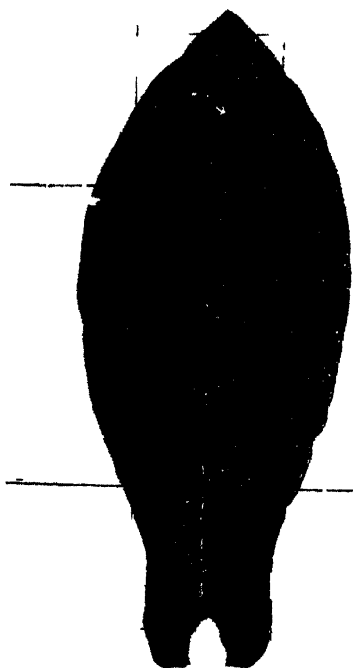


Fig. 4

Fig. 3. Typical leaf of 22035P, illustrating the lanceolate leaf-base of *N. Tabacum* var. *calycina*.

Fig. 4. Typical leaf of 22012P, illustrating the broad leaf-base type of *N. Tabacum* var. *macrophylla*.

of the whole. In two cultures petioled forms did not appear at all. They found that the indentation of the leaf-base and width of leaf were inherited independently and that environment affected both. They, too, emphasize the intermediacy of F_1 and the extreme variability of F_2 . Selections from the F_2 gave cultures which differed in their range of variation, diminishing with further selection. They did not advance any factorial explanation for their results.

EXPERIMENTAL EVIDENCE

Analysis of the leaf-base differences was somewhat complicated because of the evident presence of modifying factors affecting the development of the petiole and auricles. However, though in some cases F_2 was extremely complex, it was possible to distinguish certain forms as centers of distribution and by studying F_3 progenies to arrive

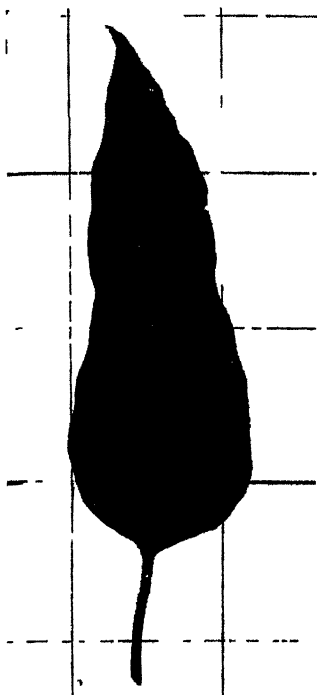


Fig. 5

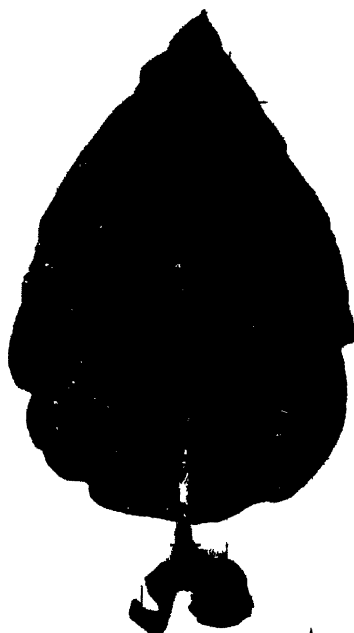


Fig. 6

Fig. 5. Typical leaf of 22027P, illustrating the petioled leaf-base type of *N. Tabacum* var. *angustifolia*.

Fig. 6. Typical leaf of 22067P, illustrating the short-petioled type of the F_1 hybrid.

at a more satisfactory idea of the significance of particular F_2 types. Several crosses were also made among the different leaf-base types of the pure varieties and these greatly facilitated formulation of a valid Mendelian explanation.

The first formed leaves (two or three in number), which remain on the ground, generally do not possess the characteristic shape of the other leaves and they had better be left out of account in classifica-

tion. In some types all of the remaining leaves, including the inflorescence leaves, are of the same form and differ only in size, while in others the inflorescence leaves are generally quite different from the lower leaves. Petioled plants generally have petioled inflorescence leaves and it is only among the non-petioled and constricted types that difficulty of classification arises. To facilitate classification petioled and short-petioled are classified together as petioled, and lanceolate and broad as sessile.

When constricted is crossed with lanceolate, F_1 is short petioled, as shown in figure 6. These results definitely demonstrate that lanceolate and broad, although both of a sessile type, are genetically distinct, for broad \times constricted gives a broad F_1 . F_2 , as shown in Table 12, yields petioled, sessile, and constricted segregants in a ratio of approximately 9:6:1. Segregants of the petioled class were variable as to length of petiole and those of the sessile class were of both broad and lanceolate types. Taken together, these results indicate that lanceolate contains the factor S , and also, A ; so that its genetic formula is $SSAA$. Since constricted is $ssaa$, F_1 short-petioled must be $SsAa$, which, because of our inability to distinguish between lanceolate and broad F_2 segregants, seems to give rise to an F_2 of 9 petioled: 6 sessile: 1 constricted.

This analysis is also in agreement with the results of backcrossing F_1 to lanceolate and *sylvestris*, as shown in Table 13. In the former, backcross segregation in the ratio of 1 petioled: 1 lanceolate is expected, and the results are in agreement. In the latter, segregation in the ratio of 1 petioled: 3 sessile is obtained because *sylvestris* acts like a broad type, $ssAA$, as shown by the F_1 constricted-*sylvestris* hybrid which is broad, although considerably narrower at the base than true broad types.

TABLE 12
 F_2 SEGREGATION FOR LEAF-BASE. P_1 = CONSTRICTED \times LANCEOLATE;
 F_1 SHORT-PETIOLED ($SsAa$) SELFED

Garden Number	Petioled	Sessile	Constricted
23166	55	33	9
182	54	44	3
183	44	36	3
24251	48	35	5
259	54	41	5
263	53	37	7
Total	308	226	32
Calculated, 9:6:1	318.4	212.2	35.4

TABLE 13
SEGREGATION FOR LEAF-BASE IN BACKCROSSES. F_1 SHORT-PETIOLED (*SsAa*)

Parentage	Garden Number	Petioled	Sessile
F_1 short-petioled \times lanceolate	23164	47	52
	167	48	51
	180	50	47
	181	40	40
	186	44	44
	187	41	55
	24253	24	25
	254	19	30
	261	26	23
	262	24	26
	265	20	30
	266	20	24
Total		403	447
Calculated, 1:1		425	425
F_1 short-petioled \times <i>sylvestris</i>	23184	6	31
	190	20	54
	191	34	66
	192	24	45
	24252	12	31
	260	13	31
	264	12	38
Total		121	296
Calculated, 1:3		104	313

TABLE 14
SEGREGATION FOR LEAF-BASE. POPULATIONS FROM SELFED PETIOLED SEGREGANTS

Garden Number	Parent Number	Petioled	Sessile	Constricted
24283	23164 P_1	28	17	2
291	166 P_1	24	18	2
292	167 P_2	24	21	3
294	166 P_{20}	26	12	2
296	166 P_{17}	23	18	4
303	167 P_{60}	27	16	3
310	186 P_{10}	20	28	2
Total		172	130	18
Calculated, 9:6:1		180	120	20
24285	23164 P_{10}	35	8	----
288	164 P_{10}	37	11	----
290	166 P_1	34	14	----
306	186 P_6	37	12	----
307	186 P_7	24	19	----
Total		167	64	----
Calculated, 3:1		173.25	57.75	----
24295	23166 P_{20}	44	----	----

This analysis has been tested further by growing selfed progenies of a number of selections of the different types. Table 14 contains a record of segregation in the progenies of selfed petioled plants. The progenies were of three kinds as expected: (1) those which conformed to a ratio of 9 petioled: 6 sessile: 1 constricted; (2) those segregating into 3 petioled: 1 lanceolate; and (3) those breeding true for the petioled condition. In addition eleven progenies were grown from selfed sessile plants. These progenies contained a total of 509 plants all of which were of the sessile types. One constricted selection yielded a progeny consisting of 48 plants, all constricted.

CONCLUSION

From these facts and those previously determined by others, the author suggests that the two pairs of leaf-base factors, *S-s*, and *A-a*, have the following expressions in various combinations:

SSAA, the lanceolate type of *apetala*, *calycina*, and Klebs-normal

SSaa, the petioled type of *angustifolia*

ssAA, the broad type of *macrophylla*, and *sessilifolia*

SsAa, the short-petioled type of F_1

ssaa, the constricted type of *purpurea*, *auriculata*, and enlarged *auriculata*

The character expression and genetic behavior of heterozygous genotypes is shown in Table 15.

TABLE 15
PHENOTYPIC EXPRESSION AND GENETIC BEHAVIOR OF VARIOUS HETEROZYGOUS
LEAF-BASE GENOTYPES

Genotype	Phenotype	Genetic Behavior
<i>S s a a</i>	Petioled	3 petioled: 1 constricted
<i>S s A a</i>	Short-petioled	9 petioled: 6 broad and lanceolate: 1 constricted
<i>s s A a</i>	Broad	3 broad: 1 constricted
<i>S S A a</i>	Petioled	3 petioled: 1 lanceolate
<i>S s A A</i>	Lanceolate	3 lanceolate: 1 broad

The writer's results agree in general with those of Setchell, Goodspeed, and Clausen (1922) with the exception that these authors apparently failed to recognize a genetic distinction between broad-base and lanceolate leaf types. In table 7, p. 493, they give *SSLLAA*, *SsLLAA*, and *SSLlAA* as petioled genotypes, whereas the present investigation demonstrates these genotypes to be of the lanceolate

type. In a number of instances these authors obtained progenies from petioled segregants which exhibited a distribution of 3 petioled: 1 broad. These broad segregants, according to the analysis here proposed, must have been of the lanceolate type.

They further distinguish between a short-petioled type, *latifolia*, and the long-petioled condition of *stenophylla*. This distinction seems to be well established, for constant *latifolia* derivatives were obtained and behaved as simple recessives to long-petioled forms, which demonstrates the existence of a factor pair, *L-l*, for length of petiole. In the present investigation considerable differences in petiole length were observed, but no attempt was made to analyze them. However, the short-petioled condition of F_1 of the genetic constitution, *SsAa*, is evidently different from the true short-petioled type which breeds true. Wing development of the petiole and leaf shapes seemed to be independent of leaf-base types, but the point was not certainly established.

LINKAGE RELATIONS

Since *N. Tabacum* has twenty-four pairs of chromosomes the chances of discovering linkage relations between genes in this species is comparatively slight. However, the data obtained in these investigations, where suitable for the purpose, were examined in order to determine whether the genes were independent or linked. Advantage was also taken of the trisomic character, "enlarged," according to the method first elaborated by Blakeslee (1922). These studies indicated that the genes, *Ap-ap*, are borne by the chromosome set involved in the "enlarged" condition; and that the factor pairs, *Aa* and *Pp*, belong to the same linkage group. In other cases the evidence indicates independent assortment.

Enlarged and *Ap-ap*

Among F_1 hybrid plants of a cross between *macrophylla* and the derivative, *auriculata*, made for the purpose of studying the inheritance of leaf-base type, Setchell, Goodspeed, and Clausen (1922) found a single plant which produced distinctly larger flowers than the rest, though identical in other respects. This plant is the source of the enlarged *auriculata* strain which has been described in detail by Clausen and Goodspeed (1924). From their genetic and cytological investigations the authors found that enlarged is a trisomic character dependent for its appearance on the presence of an extra chromosome;

viz., $2n + 1$. Enlarged selfed gives 41.1 per cent enlarged, 59.9 per cent normal, and an occasional super-enlarged. About 35.5 per cent, or nearly one-third of the functional ovules of enlarged plants, transmit the enlarged character, while only about 3.4 per cent of their pollen grains transmit it.

When apetalous is crossed with enlarged, F_1 consists of normal and enlarged plants, both of which classes produced flowers of normal shape. The normal and enlarged plants, however, differ in their subsequent genetic behavior. Normal plants gave the ratios described in previous sections of this paper, but enlarged plants from the same populations gave results which indicated that one of the genes responsible for the production of apetalous flowers was borne by the chromosome pair involved in the enlarged condition. Facts obtained through the crosses proved that this was the case, and that the gene, *Ap*, is the one represented in a triplex condition. Thus the genic constitution for enlarged is *CC Ap Ap Ap*.

Because of the great amount of material which had to be studied in a short time, the author was unable to take measurements of the flowers at the proper time for determination of the ratio of enlarged to normal within the different flower types in each population; but he is convinced from the limited number of measurements that were taken that the assumption concerning the presence of the gene, *Ap*, in a trisomic condition is justified.

Table 16 presents the data from F_2 of enlarged \times apetalous, which gave a ratio of 48 normal: 5 lacerate: 1 apetalous instead of 13:2:1, obtained from the normal-apetalous cross. Results from the backcross included in Table 17 show the difference clearly if compared with those in tables 3 and 4. When the F_1 enlarged from enlarged \times apetalous was backcrossed to Klebs-normal the resulting ratio was 8 normal: 1 lacerate instead of 3:1 (Table 4). This also indicates that the genotype, *c c Ap Ap ap*, must be normal rather than lacerate.

TABLE 16
SEGREGATION FOR FLOWER FORM IN ENLARGED CROSSES

Parentage	Garden Number	Normal	Lacerate	Apetalous
(Enlarged \times apetalous),	23183	73	7	2
F_1 enlarged selfed	24263	87	10	1
Total		160	17	3
Calculated, 48:5:1		160.0	16.7	3.3
(Enlarged \times Klebs-normal),	24259	90	---	---
F_1 enlarged selfed				

TABLE 17

SEGREGATION FOR FLOWER FORM IN ENLARGED CROSSES. P_1 = ENLARGED \times APETALOUS; F_1 ENLARGED, CROSSED WITH OTHER FORMS

Parentage	Garden Number	Normal	Lacerate	Apetalous
F_1 enlarged \times apetalous	23187	44	26	17
	24266	22	17	5
Total		66	43	22
Calculated, 5:3:1		72.7	43.7	14.6
F_1 enlarged \times Klebs-normal	23180	81	14	----
	24265	44	6	----
Total		125	20	----
Calculated, 8:1		128.9	16.1	----
F_1 enlarged \times <i>sylvestris</i>	23184	34	3	----
	192	48	5	----
	24264	44	6	----
Total		126	14	----
Calculated, 8:1		124.4	15.6	----
(Enlarged \times Klebs-normal),				
F_1 enlarged \times apetalous	24262	29	18	----
Calculated, 2:1		31.3	15.7	----

In deriving the ratios three assumptions were made: (1) random assortment of the chromosomes, (2) failure of the extra chromosome to be transmitted through the pollen, and (3) transmission of the extra chromosome by one-third of the ovules.

A-a and *P-p*

Out of the several pairs of factors reported in this paper, only two exhibited linkage relations. *Purpurea*, carmine constricted, crossed with *apetala*, pink lanceolate, gave carmine short-petioled F_1 and F_2 , 52 carmine petioled: 16 carmine sessile: 3 pink petioled: 15 pink sessile: 9 carmine constricted: 0 pink constricted; i.e., 52:16:3:15:9:0, when the expected ratio is 27:18:9:6:3:1 assuming independent assortment of genes. The linkage is illustrated better in the backcross of F_1 to *apetala* and to Klebs-normal, pink lanceolate forms. The result was 87 carmine petioled: 6 carmine sessile: 8 pink petioled: 96 pink sessile; i.e., 87:6:8:96, when the expected ratio is 1:1:1:1. It is evident from these results that *A-a* and *P-p* exhibit linkage relations, for the segregation observed in this population depends upon these factors. The percentage of crossing-over is comparatively low, 7.5 per cent calculated from the backcross data.

INDEPENDENT ASSORTMENT

The following data have been obtained from backcrosses involving other pairs of factors:

A (carmine normal \times pink Klebs-normal) $F_1 \times$ pink apetalous

17 carmine normal	
8 carmine lacerate	Expected ratio
12 pink normal	1:1:1:1
13 pink lacerate	

B (constricted normal \times lanceolate Klebs-normal) $F_1 \times$ lanceolate apetalous

9 petioled normal	
14 sessile normal	Expected ratio
13 petioled lacerate	1:1:1:1
11 sessile lacerate	

C (normal with pink filament \times Klebs-normal with green filament)
 $F_1 \times$ apetalous with green filament

20 normal with green filament	
9 normal with pink filament	Expected ratio
18 lacerate with green filament	3:1:3:1
5 lacerate with pink filament	

These results indicate independent assortment of (A) $Ap-ap$ and $P-p$; (B) $A-a$ and $Ap-ap$; and (C) $P-p$ and $G-g$, but the results are not sufficiently extensive to preclude the existence of weak linkage.

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SUMMARY

1. Normal, calycine, Klebs-normal, lacerate, and apetalous flower forms were found to exhibit the following Mendelian differences:

$CC Ap Ap$ = normal

$cc Ap Ap$ = calycine

$c^a c^a Ap Ap$ = Klebs-normal

$c^a c^a Ap ap$ = lacerate

, $c^a c^a ap ap$ = apetalous

2. Two pairs of factors account for the relation existing between red, light pink, and white flower colors. A third pair of factors is necessary to account for carmine.

3. The epistatic complementary factor G with the flower color factor P produces pink filament color. If one or both are represented by the recessive allelomorph a light green filament is produced.

4. Lanceolate, petioled, broad, short-petioled F_1 , and constricted leaf-base types were found to exhibit the following Mendelian differences:

$SSAA$ = lanceolate

$SSaa$ = petioled

$ssAA$ = broad

$SsAa$ = short-petioled F_1

$ssaa$ = constricted

5. The factor pair $Ap-ap$ exhibited trisomic inheritance ratios in association with the trisomic character, "enlarged."

6. Linkage with 7.5 per cent of crossing-over was found between the factors, $A-a$ for leaf-base type and $P-p$ for flower color.

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INHERITANCE IN NICOTIANA TABACUM
VII. THE MONOSOMIC CHARACTER, "FLUTED"

BY

R. E. CLAUSEN AND T. H. GOODSPEED

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INHERITANCE IN NICOTIANA TABACUM

VII. THE MONOSOMIC CHARACTER, "FLUTED"

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Our investigations of inheritance in *Nicotiana tabacum* to date have demonstrated that this species is not infrequently subject to chromosomal aberrations, and that it presents a combination of advantageous features extremely favorable for a study of such phenomena. These chromosomal aberrations appear spontaneously as mutants in pure lines, in F_1 hybrids, and in populations segregating for Mendelian characters. One of the forms (cf. Clausen and Goodspeed, 1924) thus discovered, "enlarged," which is characterized by an increase in flower size, has been shown to be a trisomic ($2n+1$). This paper is devoted to a second form called "fluted," which has been found on cytological examination to be a monosomic ($2n-1$).

DESCRIPTION OF FLUTED

Fluted, like enlarged, is most readily recognized by its effect upon flower size. As may be seen by reference to table 1, length of flower is decreased from 50.66 to 41.63 mm. and spread from 37.95 to 33.70 mm. The wide range of size in both fluted and normal classes is due to the fact that three progenies, which were not necessarily homogeneous for other flower size factors, were grouped to form the table. In otherwise homozygous populations both groups will doubtless exhibit much more restricted ranges.

Fluted exhibits other features, some of which may be appreciated by examination of plates 1 and 2, which make it a much more distinct and easily recognizable form than enlarged. The limb of the flower generally does not open so flat as that of the normal flower but remains, especially at first, somewhat folded, like a fluted funnel,

TABLE 1

CORRELATION TABLE OF LENGTH AND SPREAD OF COROLLA IN PROGENIES
OF FLUTED ♀ × NORMAL ♂

Data from three populations grouped; namely, 25154, 25161, and 25162.*

The group above the line represents the fluted and that below
the line the normal class

	Spread in millimeters														Total
	30	31	32	33	34	35	36	37	38	39	40	41	42	43	
38	1					1							.		2
39			2	2											4
40		2	4	1	1	4	1								13
41	1		3	2	1	7	2		1						17
42	2	1	5	4	9	1	1	1							24
43		1	1	1	3	2	5								13
44		1		1	2	1			1						6
45					1	2									3
46															
47					1	1		1							3
48							3		2						5
49							2	2	2	2					8
50			1		1		4	1	1		1	1		1	11
51					1			2		5	4	1	1		14
52						1	1	5	2	5	2	2			18
53							1				1				2
54										1		1			2
55										1					1
Total	4	5	16	11	20	20	20	12	9	14	8	5	1	1	146

* One enlarged reappearance, flower measurements 60 × 42, omitted from the table and from computations based upon it.

which accounts for the name we have given it. In fluted flowers the stamens are conspicuously shorter than the pistil, whereas in normal flowers they are of approximately the same length. Anthesis in fluted is delayed until well after the flower opens. Aside from the flowers, fluted plants are not conspicuously different from normal ones. The leaves appear to be somewhat smaller and coarser, and leaves and branches appear to be borne somewhat more erectly. Fluted may possibly be somewhat less vigorous than normal, but this difference is very slight.

Fluted sets seed well when hand pollinated with its own pollen, or in crosses of the type, fluted ♀ × normal ♂ or normal ♀ × fluted ♂; but when a portion of an inflorescence is merely bagged, as is the common practice in obtaining selfed seed in *N. tabacum*, it very often fails to produce any seed at all or only an occasional capsule. The

pollen of fluted often exhibits a high percentage of aborted grains, but since normal plants of *N. tabacum* also do so, little significance can be attached to this fact. Duplicate counts were made of pollen abortion in five fluted and five normal plants of 25164, aceto-carminic being used as a staining medium. In the fluted group the range of percentage of aborted grains was from 9.2 to 28.3; in the normal group from 1.7 to 43.8.

OCCURRENCE OF FLUTED

In late years as a part of the routine examination of cultures it has been our custom to measure length and spread of a representative flower of each plant. This practice has the effect of enforcing a more careful scrutiny of each plant and has led to the detection of a number of deviant types which might otherwise have been overlooked.

Attention was first directed to fluted in 1923. In 23073, a *tabacum* culture of F_1 *purpurea* ♀ × *calycina* ♂, one plant, P50, exhibited a flower length of 40 mm., although the rest of the plants ranged from 47 to 51 mm. In another *tabacum* culture, 23075, grown for the purpose of establishing an auriculate leaf base race identical in all other respects with *purpurea*, one plant bore flowers measuring 43 mm. in length, although the other plants in the culture ranged from 47 to 54 mm. These two plants very evidently were not strays, as subsequent tests proved, and they exhibited other features which we have since learned to recognize as characteristic of fluted. In the season of 1924 one additional spontaneous reappearance of fluted was noted, and proved by trial in the following year. In addition we had growing in that season a *tabacum* culture of 176 plants, 24230, from *purpurea* ♀ × haploid *macrophylla* ♂. Almost all of these plants were normal diploids of the characteristic F_1 type, but two had exceptionally small flowers. They were subjected to test in the following season and proved to be fluted.

In the season of 1925, by which time we had become familiar with the characteristic features of fluted, a systematic search was made in all available *tabacum* cultures for reappearances. Flowers were measured on every plant in 45 cultures, mostly of 50 plants each. Fifteen reappearances were detected among 2312 plants. One population of 49 plants contained two fluted individuals, the other 13 instances occurred as single plants in the corresponding populations. Reappearances were discovered in pure lines and in F_1 and F_2

progenies. In view of the high ratio of reappearance, about 1:150, it is doubtful whether we can consider the appearance of two fluted plants among the progeny of diploid ♀ × haploid ♂ as evidence of production of functional 23-chromosome gametes by a haploid.

GENETIC FEATURES OF FLUTED

N. tabacum is so easily manipulated in genetic experimentation that it is very readily possible to determine the characteristic features of transmission of any type and of its relations to other previously studied types. Although these studies have not yet been completed for fluted, the data collected to date do establish the main features of transmission and of relation to a few other types.

TABLE 2

PROGENIES FROM FLUTED ♀ × NORMAL ♂, FOR DETERMINATION OF THE RATIO OF TRANSMISSION OF FLUTED THROUGH OVULES

Garden No.	Total	Fluted	Per cent	Garden No.	Total	Fluted	Per cent
24137	50	33	66.0	25160	49	37	75.5
139	50	27	54.0	161	49	26	53.1
165	50	29	58.0	162	50	29	58.0
25150	50	37	74.0	165	49	32	65.3
151	49	22	44.9	166	50	29	58.0
152	50	31	62.0	172	50	27	54.0
153	50	33	66.0	173	49	25	51.0
154	49	26	53.1
Total	744	443	59.5

Female gametic transmission ratio.—The data from 15 crosses of fluted ♀ × normal ♂ have been collected in table 2. The percentage of fluted individuals in these progenies ranged from 44.9 to 75.5 per cent, with an average of 59.5 per cent for the entire group of 744 plants. The proportion of functioning 23-chromosome gametes is, therefore, significantly greater than 50 per cent, a fact to which we shall return in the discussion.

Male gametic transmission ratio.—Only five progenies have as yet been grown from normal ♀ × fluted ♂. As shown by the data in table 3, two of these progenies contained no fluted individuals, and only 7 fluted plants were obtained in the entire group of 249 plants. Moreover, the apparent percentage of pollen transmission, 2.8, should

be corrected for spontaneous reappearance, so that probably not more than 2.0 per cent of the functioning pollen grains are of the 23-chromosome class.

TABLE 3

PROGENIES FROM NORMAL ♀ × FLUTED ♂, FOR DETERMINATION OF THE RATIO OF TRANSMISSION OF FLUTED THROUGH POLLEN GRAINS

Garden No.	Total	Fluted	Per cent
25155	50	4	8.0
156	50	0	0.0
157	50	2	4.0
158	49	1	2.0
159	50	0	0.0
Total	249	7	2.8

Fluted, self-fertilized.—Data on the proportion of fluted plants in self-fertilized progenies are contained in table 4. While not very extensive, these data show a wide variation in percentage of fluted in selfed progenies; and the average, 42.6, falls far short of that obtained from fluted ♀ × normal ♂. While fluted is certainly not conspicuously less vigorous than normal, it may be significant that the two lowest

TABLE 4

PROGENIES FROM FLUTED SELF-FERTILIZED

Garden No	Total	Fluted	Per cent
24136	50	23	46.0
138	49	25	51.0
164	50	19	38.0
25163	50	29	58.0
178	50	10	20.0
Total	249	106	42.6

percentages, those from 24164 and 25178, occur in a line which had been self-fertilized for six generations prior to 1924, so that these progenies are F₇ and F₈, respectively.

A comparison of parallel progenies of tables 2 and 4 lends support to this conclusion. The parallels are 24136 with 24137, 24138 with 24139, and 24164 with 24165. In the first two pairs of cultures the same mother plant was selfed to give the progeny recorded in table 4, and crossed with *N. tabacum* var. *purpurea* to give that in table 2.

In the third the mother plant which was selfed to give 24164 (table 4) was crossed with *sylvestris* to give 24165 (table 2). As may be seen by reference to table 5, the potential production of functional 23-chromosome ovules in these plants did not differ significantly from that of the entire group recorded in table 2.

TABLE 5
COMPARISON OF PARALLEL PROGENIES OF FLUTED SELF-FERTILIZED AND
FLUTED ♀ × NORMAL ♂

Garden number of fluted parent	Fluted x self	Fluted ♀ x normal ♂
	per cent fluted	per cent fluted
23073P50	46.0	66.0
075P17	51.0	54.0
127P14	38.0	58.0
Mean	45.0	59.3

Relation of fluted to enlarged.—Since the monosomic, fluted, and the trisomic, enlarged, both reappear comparatively frequently in our cultures, and since in both cases the chromosome involved is obviously one which contains an excess of factors tending to increase flower size, it was possible that they represented complementary products of non-disjunction in the same chromosome set. Under this assumption, however, the production of an enlarged fluted combination should be impossible, for the union of a 23-chromosome fluted with a 25-chromosome enlarged gamete would merely give the normal form. Ordinarily it would be difficult to put this matter to a test, because both enlarged and fluted exhibit such low ratios of pollen transmission. However, the tetrasomic, superenlarged, produces a high proportion of functioning 25-chromosome pollen grains, so it was utilized for the purpose. A single progeny of 25164, from fluted ♀ × superenlarged ♂, gave the following results:

7 normal
21 enlarged
2 fluted
19 enlarged fluted

Enlarged fluted flowers are practically the same size as normal ones, but they are readily distinguishable by reason of the associated features characteristic of fluted. Obviously the production of enlarged and fluted depends upon non-disjunction in different pairs of chromosomes.

Relation of fluted to Mendelian factors.—As Bridges (1921) has shown for haplo-IV in *Drosophila melanogaster*, monosomic forms may be used advantageously for a determination of linkage groups and also of linkage relations within a group. Applying the idea to fluted, a cross of the type, fluted dominant ♀ \times normal recessive ♂, should give fluted recessive and normal dominant offspring, if the factors in question are borne by the chromosome set involved in the production of fluted; if not, both fluted and normal offspring should be of the dominant type. Unfortunately, we are not yet in possession of a sufficiently large number of well-defined Mendelian characters to make a complete test of this matter; but trials with the following pairs of characters have given negative results:

<i>A-a</i>	broad vs. auriculate leaf base
<i>C-c</i>	normal vs. calycine flower shape
<i>G-g</i>	colored vs. green filament
<i>P-p</i>	carmine vs. pink flower color
<i>R-r</i>	pink vs. red flower color
<i>W-w</i>	colored vs. white flowers

Since *A-a* and *P-p* are known to be members of the same linkage group (cf. Kelaney, 1925), at most only five linkage groups of the expected twenty-four are represented in these trials.

Fluted sylvestris-tabacum hybrids.—As we have pointed out heretofore (Goodspeed and Clausen, 1917), the normal result of crossing *sylvestris* with any variety of *tabacum* is the production of a sterile F_1 hybrid reproducing the characters of its *tabacum* parent on an enlarged scale. This type of cross is often very useful for analytical purposes, for it is sometimes possible to avoid synthesis of double recessives necessary for backcrossing by using *sylvestris* instead.

From crosses of the type, fluted ♀ \times *sylvestris* ♂, progenies are obtained which are sharply divided into two classes: fluted *sylvestris-tabacum* and normal *sylvestris-tabacum* hybrids. The distinction between the fluted and normal *sylvestris-tabacum* hybrids is more pronounced than that between the corresponding *tabacum* forms cf. pls. 2 and 3). The difference in flower size is very much exaggerated, as may be seen by reference to the measurements of corolla length recorded in table 6. The difference in mean corolla length is nearly 15 mm. instead of about 9 mm. as in the corresponding *tabacum* classes. As a matter of fact the corolla length of the fluted *sylvestris-tabacum* hybrid is only slightly greater than that of fluted *tabacum*, so that in this respect the general enlargement observed in F_1 *sylvestris-tabacum* hybrids does not occur.

This exaggeration of the difference between the fluted and normal classes is also shown in other respects in which there is at most only a slight difference between the corresponding *tabacum* classes. The fluted plants as a whole have a more distinctive appearance. The branches and leaves are borne more erectly, the leaves are smaller and coarser in texture, and the entire plant is less robust. The average height of the fluted hybrids is about two feet less than that of the normal ones.

TABLE 6

DISTRIBUTION OF TUBE LENGTHS IN FLUTED AND NORMAL *sylvestris-tabacum* HYBRIDS IN 25160 = FLUTED *tabacum* ♀ × *sylvestris* ♂

Class	Tube lengths in millimeters												Mean
	39	40	41	42	43	44	45	46	47	48	49	50	
Fluted	1	—	1	3	8	4	10	5	3	2	—	—	44 41
Normal	51	52	53	54	55	56	57	58	59	60	61	62	59 36
	—	—	—	—	—	—	—	2	5	2	2	—	

The most unexpected difference, however, is that in flower color. Fluted *tabacum* in association with carmine, pink, or red flower color is identical in color with the corresponding normal plants. We have shown previously (cf. Goodspeed and Clausen, 1917) that the F_1 *sylvestris-tabacum* hybrids are only very slightly lighter in flower color than the corresponding *tabacum* varieties. In fluted *sylvestris-tabacum* hybrids, however, carmine is changed to coral; pink to very light pink faintly tinged with salmon; and red to pink with a slight salmon flush. In the more precise terms of the Ridgway scale the carmine F_1 is rose-red, while fluted F_1 is geranium pink. The former belongs in the series of reds modified with violet; the latter in that of reds modified with orange.

These results led us to conclude that the contrast of fluted with normal in *tabacum* is not equivalent to that of these two classes of *sylvestris-tabacum* hybrids. Goodspeed (1923) has shown that in meiosis in the F_1 *sylvestris-tabacum* hybrid the 12 *sylvestris* chromosomes pair with 12 *tabacum*, leaving 12 unpaired *tabacum* chromosomes. If we designate the chromosome concerned in the fluted form as F, and assume that this chromosome has no homologue in *sylvestris*, then the fluted *sylvestris-tabacum* hybrid is nullo-F, i.e., no F-chromo-

some is present in it. If this assumption is correct, as the cytological evidence presented below indicates, the corresponding comparison in *tabacum* would necessitate the production of the nullo-F class, i.e., production of a *tabacum* with both members of this pair of chromosomes eliminated, instead of only one as is the case in fluted. This may prove to be a difficult matter, but some weight may be attached to the argument from consideration of the bud variation described below.

A bud variant in fluted.—Late in the season a fluted carmine *tabacum* plant, 25150 P1, produced a single large branch, the flowers of which were coral in color, almost identical with those described for the fluted *sylvestris-tabacum*. Since this flower color is one which we have never before seen in *tabacum*, the only reasonable explanation that we can offer for its appearance is that the epidermal tissues of this branch have become nullo-F through loss of the single F-chromosome in fluted. As we have shown in a previous paper (Clausen and Goodspeed, 1923), a change in flower color of this character necessitates only a change in the genetic constitution of the epidermal layer. Whether the change in this instance involved deeper-lying tissues has not yet been determined. If this explanation is correct, nullo-F evidently represents a viable condition in conjunction with haplo-F tissue and probably would also be viable independently.

THE CYTOLOGICAL SITUATION IN FLUTED

The following preliminary description of the chromosome situation in fluted is based upon studies of both aceto-carmin preparations and fixed material. With the exception of figure 1, the drawings were in all cases made from aceto-carmin preparations, some from crushed and some from uncrushed pollen mother cells.

The diakinesis shown in figure 1 is characteristic of a number which were studied and indicates that the chromosome garniture of fluted consists of 23 bivalents and a single univalent. The distinction between the clearly associated bivalents and the single univalent was sharp. In early first metaphases 23 large and a single small chromosome occurred; later metaphases being difficult to count accurately, apparently because of a somewhat premature disjunction of one of the bivalents. The univalent usually does not lie in the equatorial plate

so that many of the heterotypic metaphases in side view show it clearly detached from the bivalents. In polar view it is distinguishable by reason of its smaller size and difference in focus as compared with the bivalent chromosomes. These matters are illustrated in figure 2, *a* and *b*. As indicated in figure 2 *c*, late first anaphases often show the single univalent lagging in the spindle and apparently in process of division, the bivalent partners having practically completed their passage to the poles. In most cases, however, a division of the univalent does not occur, as evidenced by counts of a considerable number of second divisions which showed 23 chromosomes in one and

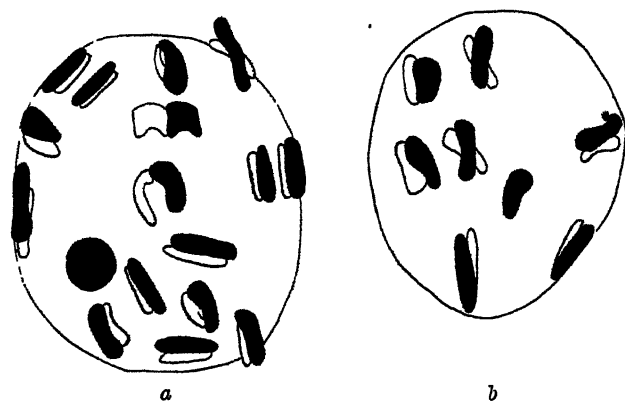


Fig. 1. Diakinesis in p.m.c. of a fluted plant.
a, 16 bivalents; *b*, 7 bivalents and 1 univalent.

24 in the other metaphase (fig. 2 *d*). In second anaphases the univalent often lags in a similar fashion; its division, on the other hand, is probably completed in most cases to produce in each tetrad two microspore nuclei each with 24 chromosomes.

It is often found, however, on close examination of the late tetrad condition that one or more of the microspores may contain a small nucleus in addition to the normal one. These micronuclei usually appear as closely appressed against or in the near neighborhood of the larger nucleus. They probably represent failure of the univalent chromosome to be fully incorporated in the major products of the two meioses. If this interpretation is correct and if these micronuclei eventually degenerate, as Kihara (1924) and Watkins (1925) assumed for wheats of unbalanced chromosomal constitution obtained from interspecific hybridization, then obviously the proportion of 23-chromosome gametes will be increased at the expense of the 24-

chromosome type. In order to place this matter on a quantitative basis counts were made of the number of microspores containing micronuclei in 122 tetrads with the following results:

Number of micronuclei	Number of tetrads
0	60
1	44
2	17
3	1
Total	122

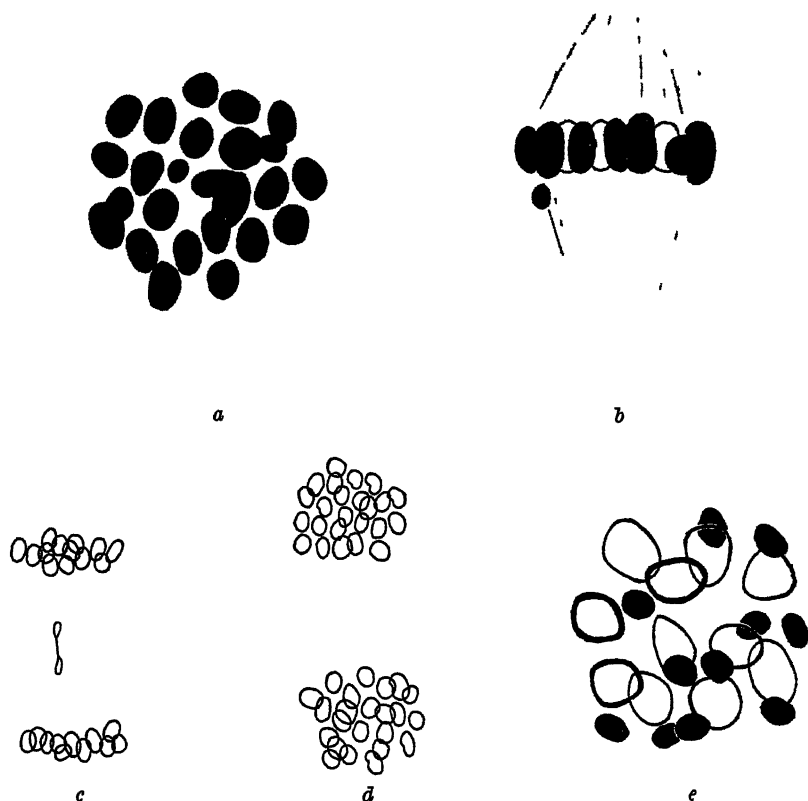


Fig. 2. P. m. c. *a*, heterotypic metaphase of fluted, polar view, 23 larger, bivalent chromosomes and near the center the single, smaller univalent. *b*, the same in side view, showing the position of the univalent off the equatorial plate of bivalents. *c*, a late heterotypic anaphase of fluted, showing the single univalent lagging and apparently dividing. *d*, homotypic metaphase, 23 chromosomes in one plate and 24 in the other. *e*, heterotypic metaphase of the F_1 fluted sylvestris-tabacum hybrid, showing 11 of the 12 large bivalent chromosomes in an equatorial plate and the 11 smaller univalents lying scattered above and below the plate.

Possibly primary non-disjunction in some other pair of chromosomes accounts for the tetrad which contained three microspores with micronuclei, and the same phenomenon may, of course, be responsible for the production of some of the other microspores with micronuclei. Assuming, however, that each micronucleus represents half of the univalent chromosome, which has failed of incorporation in the nucleus by lagging, then the numbers of 23- and 24-chromosome nuclei will be 320 and 164, respectively, omitting from the computations the tetrad containing three microspores with micronuclei; or the percentage of 23-chromosome gametes will be 65.5, instead of 50 as might be expected, if no such irregularities occur. This number agrees fairly well with the actual excess of fluted plants obtained in the progenies of fluted ♀ \times normal ♂ (table 2), particularly if some allowance be made for a somewhat reduced viability in fluted. Possibly, therefore, lagging of the univalent chromosome is responsible for the observed excess of fluted plants in these progenies, although we have not yet studied this phenomenon in megaspore meiosis.

In some instances the group of microspores consists of four of normal size plus a small microcyte. Apparently a microcyte is formed around the micronucleus when the latter happens to lie near the surface of the pollen cell. It is possible, on the other hand, that microcyte formation may only follow cases in which a lagging univalent in the first division remains permanently in the plasma. Counts of 173 pollen groups disclosed the presence of eight which contained one microcyte each, so that this phenomenon is much less frequent than simply the formation of micronuclei, and is not sufficiently common to account alone for the observed distortion in transmission ratios.

In the normal F_1 *sylvestris-tabacum* hybrid, as shown by Goodspeed (1923), there are twelve well-defined bivalent chromosomes and twelve univalents. In favorable preparations of first metaphases the twelve bivalents lie in a compact equatorial plate and the twelve univalents, usually distinctly smaller than the bivalents, lie scattered above and below this group, as seen in polar view. In similar preparations from the F_1 fluted *sylvestris-tabacum* hybrid, the equatorial plate likewise consists of twelve well-defined bivalent chromosomes; and in a few particularly good preparations it has been possible to count eleven univalents scattered above and below this plate (cf. fig. 2 e). Apparently, therefore, the unpaired chromosome in fluted is derived from one of those pairs of chromosomes from which the univalent chromosomes of the normal F_1 *sylvestris-tabacum* hybrid come.

DISCUSSION OF RESULTS

Not many instances of monosomic forms have been recorded; nor have those discovered been utilized genetically to the fullest possible extent. Bridges (1921) has shown how the monosomic, haplo-IV in *Drosophila melanogaster* may be employed in determinations of group and linkage relations; and a similar utilization should be possible in other species in which they are marked by recognizable morphological features. Kihara (1924) has shown that they may be derived in wheat from crosses between emmer ($n=14$) and spelt ($n=21$) forms; but apparently those he obtained are not morphologically distinguishable from normals. However, he has shown that the corresponding 40-chromosome forms may be obtained in small numbers in the selfed offspring of 41-chromosome plants. These 40-chromosome forms are very distinct, dwarf or semi-dwarf types, which have the advantage of constancy. It should be possible to employ them in the same way as monosomics for genetic studies. Winge (1924) has also shown that some aberrant wheat forms which occur spontaneously are monosomics; but in these instances additional subsidiary hypotheses appear to be necessary to account for the observations. In *Datura* a monosomic condition has been established by bud variation; but apparently ($n-1$) gametes are not viable (cf. Blakeslee and Belling, 1924*a*). The fact that they may be derived from hybrids between species differing in chromosome number, however, makes it possible to obtain them readily without simply waiting for their spontaneous appearance as would be necessary otherwise. There are, however, obvious limitations to this method, since, theoretically at least, only monosomics of the sets which furnish the univalent chromosomes of the F_1 hybrids may thus be established. Theoretically it should be possible to obtain a complete set of them among the progeny of haploids; but the *Datura* haploid, at least, appears to give only normal diploid offspring (cf. Blakeslee and Belling, 1924*b*).

Despite the fact that the two cases are not directly comparable, an interesting parallel is shown in the female gametic transmission ratios of the trisomic enlarged and the monosomic fluted, as may be seen from the following:

$$\begin{array}{l} \text{enlarged } \varnothing \times \text{normal } \sigma^7 = 35.5 \text{ enlarged} + 64.5 \text{ normal} \\ \text{fluted } \varnothing \times \text{normal } \sigma^7 = 40.5 \text{ normal} + 59.5 \text{ fluted} \end{array}$$

In both cases the proportion of gametes receiving the "odd" chromosome is evidently well below the expected 50 per cent, which probably indicates that in *tabacum* chromosome elimination resulting from failure of inclusion of the "odd" chromosome in the eventual reduced nuclei is the basis of the deviation of transmission ratios from expectation, rather than ovule abortion as proposed by Buchholz and Blakeslee (1922) to explain the comparatively low transmission ratios of trisomics in *Datura*. If this relation between the female gametic transmission ratios should prove to be of general application, it would provide a ready means of distinction between monosomics and trisomics on the basis of breeding results.

The actual female gametic transmission ratios obtained with fluted agree closely with those published by Kihara (1924) for 41-chromosome derivatives from emmer-spelt crosses. He found by actual cytological examination of tetrad formation that the unpaired chromosome is often eliminated to such an extent that the ratio of 20- to 21-chromosome nuclei was very nearly 3:2. In crosses of 41-chromosome ♀ × 42-chromosome ♂, 11 offspring had 41 and 4, 42 chromosomes, which would indicate that the same process of chromosome elimination occurs in the megaspores as in the microspores. The ratio of 3:2 based on 254 counts is almost exactly the same as our ratio of transmission determined genetically. Kihara, however, apparently found a rather high ratio of transmission in the male gametic series. From 42-chromosome ♀ × 41-chromosome ♂ 14 offspring were counted, of which 6 had 41 and 8, 42 chromosomes. His results from self-fertilized 41-chromosome plants in the main confirm this conclusion, for as Watkins (1925) has shown they are consistent with the gametic ratios of transmission separately determined, except for the fact that 40-chromosome plants are produced in far less than the expected numbers. Perhaps the highly selective action against 23-chromosome gametes in *tabacum* is merely a consequence of the long distance which the pollen tube must traverse before reaching the ovules.

In previous papers we have stated that the F_1 *sylvestris-tabacum* hybrid crossed back to *tabacum* gives among a variety of sterile types a certain small proportion of partially fertile plants of prevailing *tabacum* type, which when subjected to self-fertilization eventually gives rise to fully fertile derivatives identical with the original *tabacum* parent. We suggested that this reversion to the parental type depended upon (1) differential viability of gametes, those having complete or nearly complete *tabacum* sets being favored, and (2)

zygotic elimination of combinations which do not possess at least one *tabacum* member in each chromosome set, either through actual lethal effect upon the zygote or through production of sterile plants. Since these suggestions were based upon the assumption, since shown to be incorrect (cf. Goodspeed, 1923), that both species have 24 pairs of chromosomes, they clearly require modification in the light of present knowledge of the cytological conditions. The correct chromosome numbers are $n=12$ for *sylvestris* and $n=24$ for *tabacum*, and F_1 exhibits pairing according to the Drosera scheme, $12_{II}+12_I$. The problem is, therefore, twofold: (1) the nature of the distinction between *sylvestris* and *tabacum* homologues and the effects following replacement of *tabacum* by *sylvestris* homologues and (2) the behavior of univalent chromosomes.

The results with fluted bear definitely upon the second portion of this problem. In effect they indicate that differential functioning of pollen classes strongly favors those which have complete, 24-chromosome complexes. As a consequence any partially fertile derivative from this backcross would probably produce very few, if any, descendants which did not have at least one of each of these univalent chromosomes; and in further generations those which remain in the univalent condition would rapidly become paired up through the operation of the same selective functioning of pollen classes. Gametic elimination or selective functioning of ovule classes in these further generations is unnecessary to account for the results obtained, although it may possibly occur to a certain extent. As a matter of fact we have isolated a monosomic *tabacum* derivative from this hybrid, an account of which appears as a separate paper entitled, "Interspecific Hybridization in *Nicotiana*. III. The monosomic *tabacum* derivative, corrugated, from the *sylvestris-tabacum* hybrid," to which the reader is referred for further details.

SUMMARY

1. A form, fluted, occurring spontaneously in *N. tabacum* cultures in the frequency of about 1:150, and readily recognizable from its distinct morphological features, was found on cytological examination to be a monosomic ($2n-1$).

2. Studies of transmission showed that about 60 per cent of the functioning ovules and 2 per cent of the functioning pollen grains are ($n-1$). Selfing fluted apparently gives a somewhat lower rate of transmission than according to expectation based on these percentages. Fluted was shown to involve a different chromosome set than the trisomic enlarged, and to be independent of five other linkage groups.

3. Crosses of fluted *tabacum* ♀ × *sylvestris* ♂ give fluted and normal F_1 *sylvestris-tabacum* hybrids, the morphological differences between which are more pronounced than those between fluted and normal *tabacum*, particularly in flower color. This is believed to be due to the fact that the F_1 fluted *sylvestris-tabacum* hybrid is really nullo-F, and as such comparable to the corresponding nullo-F *tabacum* rather than to fluted which is haplo-F. Supporting evidence for this assumption is given by a coral bud variant on a fluted *tabacum* plant, which apparently arose through establishment of the nullo-F condition by elimination of the single F-chromosome in fluted.

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EXPLANATION OF PLATES

PLATE 1

Lateral inflorescences of (*A*) fluted as compared with (*B*) normal in *N. tabacum* var. *purpurea*. Note the more compact inflorescence of fluted, the shorter blossoms and the shorter stamens. The fluted character of the expanded limb of fluted may also be contrasted with the flatter expanded limb of the normal type. (Photographed by W. C. Matthews.)



PLATE 2

Typical flowers in profile and face view of (A) normal *tabacum* var. *purpurea*, (B) fluted mutant of *tabacum* var. *purpurea*, (C) normal F₁ *sylvestris-tabacum* hybrid, and (D) fluted F₁ *sylvestris-tabacum* hybrid. Note shorter tube length and decreased spread of fluted as compared with corresponding normal, late anthesis in fluted as shown in (B), and peculiar lobing of the corolla and lighter flower color of the fluted F₁ *sylvestris-tabacum* hybrid. (Photographed by W. C. Matthews.)



PLATE 3

Lateral inflorescences of (A) the fluted F_1 *syvestris-tabacum* hybrid and (B) the normal F_1 *syvestris-tabacum* hybrid. Note the more compact inflorescence, the shorter flowers and the shorter stamens, and the distinctive type of lobing of the corolla of fluted as compared with normal. Compare with plate 1. (Photographed by W. C. Matthews.)



INTERSPECIFIC HYBRIDIZATION IN NICOTIANA

III. THE MONOSOMIC TABACUM DERIVATIVE, "CORRUGATED," FROM THE SYLVESTRIS- TABACUM HYBRID

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As a result of detailed and prolonged studies of hybrids between *Nicotiana sylvestris* and varieties of *N. tabacum*, we have arrived at some definite conclusions, which may be summarized briefly as follows. When *sylvestris* is crossed with any variety of *tabacum*, a nearly completely sterile hybrid is obtained which, aside from its enhanced vigor, closely resembles its particular *tabacum* parent. Such hybrids will not produce seed by self-pollination, but they will produce a few seeds by backcrossing to either parent. The resulting progenies are very heterogeneous. It is doubtful that we have ever seen two plants among them which were exactly alike; but in the backcross to *tabacum* there are invariably a number of plants which rather closely resemble the *tabacum* parent and which give rise after a few consecutive generations of self-pollination to fully fertile derivatives which are exact duplicates, aside perhaps from a slight difference in vigor, of their original *tabacum* parent. When F_1 is backcrossed to *sylvestris*, there are produced, likewise, a few partially fertile plants which are predominantly *sylvestris* in their characters, and which give rise after a few consecutive generations of self-pollination to fully fertile derivative lines identical with pure *sylvestris* (cf. Goodspeed and Clausen, 1922). A schematic portrayal of these results is given in figure 1.

To account for this remarkable behavior, we advanced the reaction-system hypothesis (cf. Goodspeed and Clausen, 1917), the underlying assumption of which is that from an evolutionary point of view the two species have diverged so widely that exchanges of germinal material between them are incompatible with normal functioning in development. According to this conception, any zygote which does not receive a complete set, either of *sylvestris* or of *tabacum* elements, fails to develop. If the distributory mechanism of reduction functions

TABLE 1
CHROMOSOME CONTENT AND FREQUENCIES OF THE GAMETES OF THE *F₁ sylvestris-tabacum* HYBRID*
Number of *sylvestris* chromosomes

	0	1	2	3	4	5	6	7	8	9	10	11	12	Total
0														1
1											66	12	12	24
2											792	144	66	276
3											4356	792	220	2024
4									495	220	2640	2640	495	10626
5								792	5940	2640	14520	5940	792	42504
6							924	9504	32670	48400	32670	9504	924	134596
7						792	11088	52272	108900	108900	52272	11088	792	346104
8					495	9504	60984	174240	245025	174240	60984	9504	495	735471
9				220	5940	52272	203280	392040	392040	392040	52272	5940	220	1307504
10			66	2640	32670	174240	457380	627264	457380	174240	32670	5940	66	1961256
11		12	792	14520	108900	392040	731808	731808	731808	392040	14520	2640	12	2496144
12	1	144	4356	48400	245025	627264	853776	627264	245025	48400	4356	144	1	2704156
13	12	792	14520	108900	392040	731808	731808	392040	108900	14520	792	12		2496144
14	66	2640	32670	174240	457380	627264	731808	392040	32670	2640	66			1961256
15	220	5940	52272	203280	392040	392040	203280	174240	5940	220				1307504
16	495	9504	60984	174240	245025	174240	60984	9504	495					735471
17	792	11088	52272	108900	108900	52272	11088	792						346104
18	924	9504	32670	48400	32670	9504	924							134596
19	792	5940	14520	14520	5940	792								42504
20	495	2640	4356	2640	495									10626
21	220	792	792	220										2024
22	66	144	66											276
23	12													24
24	1													1
Total	4096	49152	270336	901120	2027520	3244032	3784704	3244032	2027520	901120	270336	49152	4096	16777216

Number of *tabacum* chromosomes

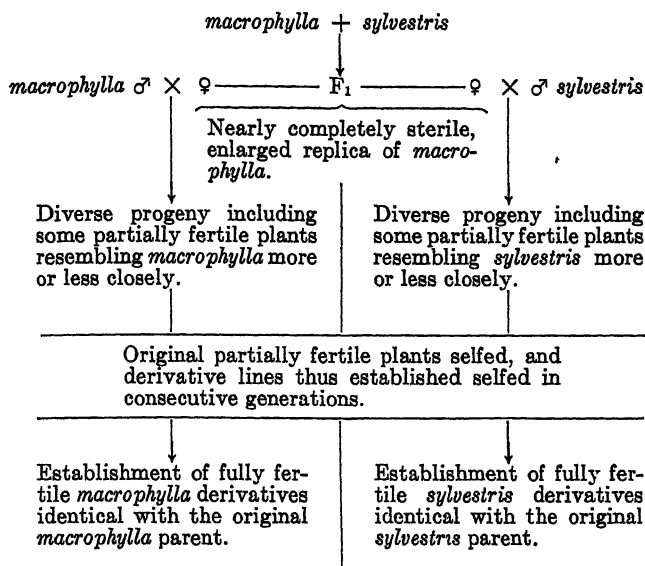


Fig. 1. Schematic portrayal of the results of crossing *sylvestris* with the *tabacum* variety *macrophylla*. The original forms emerge unmodified. The scheme will serve for other varieties of *tabacum* by substituting their names for *macrophylla*.

normally, this assumption is the only one needed in order to account for the rapid and complete return to the parental condition which has been observed in our experiments.

In our original outline of this hypothesis we assumed that the two parental species had the same chromosome number, namely, $n=24$. We have since found (cf. Goodspeed, 1923) that they differ in chromosome number; $n=12$ for *sylvestris* and $n=24$ for *tabacum*, a fact which must be taken into account in arriving at a correct explanation of the genetic features of this hybrid. The F_1 hybrid has 12 *sylvestris* and 24 *tabacum* chromosomes; and the pairing in meiosis appears to follow the *Drosera* scheme, 12 *sylvestris* with 12 *tabacum* and 12 univalent *tabacum* chromosomes, which may be represented by the chromosome formula, $12_{ST} + 12_T$. In view of this fact it is of course necessary to consider not only exchanges of germinal material between the two species, which presumably are limited to the twelve gemini of the F_1 hybrid, but also the distribution of the univalent *tabacum* chromosomes. Assuming random orientation of the 12 bivalents and random distribution of the 12 univalents, the full possibilities as respects chromosome content of the F_1 gametes are shown in table 1 with their respective frequencies. Probably the conditions assumed

are not fulfilled in the actual material, but they represent a logical point of departure for a consideration of the problem.

The evidence seems to indicate that of this potential recombination series only those gametes are functional which contain complete or nearly complete sets of *sylvestris* or *tabacum* chromosomes. For purposes of illustration assume that one of the partially fertile individuals of the *tabacum* backcross progeny arises from union of a female gamete containing $18 \text{ tabacum} + 3 \text{ sylvestris}$ chromosomes with a male *tabacum* gamete containing a full set of *tabacum* chromosomes. Its chromosome formula would then be $18_{\text{TT}} + 3_{\text{ST}} + 3_{\text{T}}$. If in the self-fertilization of such an individual all those zygotes are lethal which do not contain at least one of each kind of *tabacum* chromosome, the surviving zygotes will constitute $(3/4)^6$ or 17.8 per cent of the entire possible zygote series under the usual assumptions as to chromosome distribution. Self-pollination under these conditions obviously will operate to eliminate the *sylvestris* and to double up the univalent *tabacum* chromosomes, thus eventually producing derivatives with a full diploid set of *tabacum* chromosomes. The same line of reasoning applies *mutatis mutandis* to the establishment of pure *sylvestris* derivatives.

While the foregoing assumptions do not appear to be unreasonable, the evidence which has been presented does not by any means conclusively establish their correctness. We have therefore endeavored in our further study of *tabacum* derivatives of this hybrid to obtain additional information on two specific problems: (1) the possibility and the effect of replacing *tabacum* chromosomes with *sylvestris* homologues, and (2) the behavior of univalent *tabacum* chromosomes in these derivatives. Similar studies are in progress with *sylvestris* derivatives. These seem to be the fundamental problems concerned in this hybrid; when solved they should provide us with an interpretation, as free from speculative assumptions as possible, of its remarkable genetic features. The present paper bears definitely on the second problem outlined above. .

THE "CORRUGATED" TABACUM DERIVATIVE

The history of the particular *tabacum* derivative described in this paper runs back to 1915. In that year flowers on two plants of the F_1 *macrophylla-sylvestris* hybrid, 15F₁H38, growing in the greenhouse, were castrated and pollinated with *macrophylla* pollen. From the seeds thus obtained, a backcross progeny was grown under the garden number, 16F₁H223. Not much was done with it. It exhibited the usual diversity and a number of plants rather closely approximating *macrophylla* were partially fertile. Five of the partially fertile plants were selfed and 100 plants of each were grown in 1917. Without exception these produced red-flowering plants exclusively, but some of them were variable in other respects. The most variable progeny was 17F₂H223P7, which was particularly characterized by the presence of a distinctly abnormal class of dwarf, semi-sterile plants in addition to a normal class identical with *macrophylla* and some plants not readily placed in either of these two categories. From a cross of the parent of this culture, 16F₁H223P7, with *sylvestris* a progeny, 17F₁H224, was obtained which contained 62 red and 38 white flowering plants.

In 1918, four F_3 populations of 50 plants each were grown which were derived from three different plants of the original 16F₁H223. These were all uniform and closely approximated *macrophylla* in all their characters. Four cultures of 100 plants each were also grown of different F_2 plants crossed with *sylvestris*. Three of these consisted almost exclusively of plants identical with the normal F_1 *macrophylla-sylvestris* hybrid. The other contained 64 red, 3 pink, and 33 white flowering plants.

In 1919, the results obtained thus far were reviewed for the purpose of planning the subsequent work. Although the cultures had gone only to F_3 , it was clearly evident that the derivatives had returned almost completely to the parental *tabacum* type. Two distinct lines were continued to F_6 , but aside from an enhanced vigor, they were identical with *macrophylla*. When crossed with *sylvestris* both gave F_1 hybrids identical with the normal F_1 *macrophylla-sylvestris* hybrid. Since, however, the purpose of the investigations was not to demonstrate this return to the parental condition, which had already been adequately established, but to determine how it was accomplished,

we decided to go back to one of those plants which had yielded a solely red-flowering progeny on self-fertilization, but which gave red and white flowering plants when crossed with *sylvestris*. Since 16F₁H223P7, as noted above, gave this result, the two 1917 sowings of this plant were repeated in 1919 as 19F₂H223P7 and 19F₁H224 and subjected to closer scrutiny than was possible in 1917. The results were essentially the same; 19F₂H223P7, the selfed progeny,

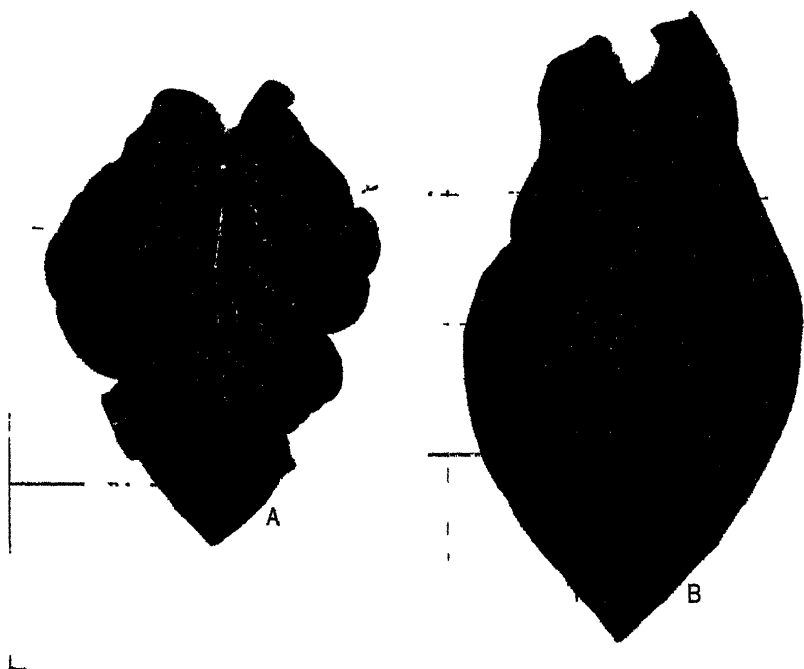


Fig. 2. Leaves of (A) corrugated, 22121P6, and (B) normal, 22121P8, segregants in the same population. The background is ruled in decimeter squares.

consisted of 72 normal, 6 slightly abnormal, and 22 distinctly abnormal plants; 19F₁H224, its progeny when crossed with *sylvestris*, consisted of 43 red- and 57 white-flowering plants.

One of the abnormal plants, 19F₂H223P7P84, was selfed and crossed with *sylvestris* in order to determine whether or not these results would be repeated. Its selfed progeny, 20084, was uniformly red-flowering and fairly definitely distributed into two classes; a normal class equivalent to *macrophylla*, the original parent, and an abnormal class like that in the parent culture. Its progeny from the

cross with *sylvestris*, 20050, consisted of two distinct classes duplicating those of the previous cross; one red-flowering and equivalent to the original F_1 *macrophylla-sylvestris* hybrid, the other white-flowering and differing in other less conspicuous features from the first class. These results led us to embark upon a detailed study of the abnormal *tabacum* derivative, which was henceforth called "corrugated" on account of its distinct leaf-type.

Corrugated differs from normal in a complex of characters. Plants are most easily separated into the two categories by examination of leaves, which have roughened, bullated surfaces in corrugated, and smooth, flat ones in normal (cf. fig. 2). Corrugated also produces slightly larger flowers than normal plants, its leaves slope downward from the stem, and its stems have shorter, thicker internodes than normal. There are also numerous other less well-defined differences which separate the two forms. The entire complex of characters acts as a unit in heredity.

The parentage of the cultures reported in this paper has been brought together in table 2 in such a way that it is possible for the reader, by referring to it, to determine the entire pertinent ancestry of any culture or series of cultures reported in the text or in other tables. We have come to the conclusion from the cytological studies reported below that corrugated is a monosomic ($2n-1$) *tabacum* derivative. The evidence presented below has been so arranged as to show its relation to this conception.

CYTOLOGICAL FEATURES OF CORRUGATED

The cytological features of corrugated are so nearly identical with those of fluted (cf. Clausen and Goodspeed, 1926) that statements applying to it may be repeated without change for corrugated. Favorable preparations of pollen mother cells in first metaphase show 23 bivalent chromosomes with a single smaller univalent, which does not take up any very characteristic position (cf. fig. 3 a). As with fluted in side view the unpaired chromosome is often seen to lie outside the plane of the equatorial plate (cf. fig. 3 b) and it frequently lags on the spindle after the bivalent partners have separated and are passing to the poles. In a few cases counts of both metaphase plates in the homotypic division have shown 23 chromosomes in one plate and 24 in the other (cf. fig. 3 c). Cytological studies for this form have not been pursued beyond a determination of its chromosome number.

TABLE 2

PARENTAGE AND DESCRIPTION OF VARIOUS CULTURES GROWN IN THE
INVESTIGATIONS OF THE BEHAVIOR OF CORRUGATED

Garden number	Parentage	Description
15F ₁ H38	14 22/07 x 69/07.....	<i>macrophylla</i> x <i>sylvestris</i>
16F ₁ H223	15F ₁ H38 x 22/07.....	F ₁ <i>macrophylla-sylvestris</i> x <i>macrophylla</i>
19F ₂ H223P7	16F ₁ H223P7.....	Partially fertile backcross plant
20050 084	19 69/07P1 x 19F ₂ H223P7P84.... 19F ₂ H223P7P84.....	<i>sylvestris</i> x F ₂ corrugated F ₂ corrugated selfed
21072	20084P1 x 015P1.....	F ₂ normal x white <i>tabacum</i>
073	20084P22.....	F ₂ corrugated selfed
074	20084P22 x 19 69/07.....	F ₂ corrugated x <i>sylvestris</i>
075	20084P22 x 015P7.....	F ₂ corrugated x white <i>tabacum</i>
077	20084P14 x 19 69/07.....	F ₂ normal x <i>sylvestris</i>
078	20084P14 x 015P5.....	F ₂ normal x white <i>tabacum</i>
079	20084P15.....	F ₂ corrugated selfed
080	20084P15 x 19 69/07.....	F ₂ corrugated x <i>sylvestris</i>
081	20084P15 x 015P3.....	F ₂ corrugated x white <i>tabacum</i>
20092	21073P8.....	F ₄ corrugated selfed
093	21050P4 x 073P8.....	<i>sylvestris</i> x F ₄ corrugated
095	21073P5 x 050P17.....	F ₄ corrugated x <i>sylvestris</i>
121	21073P6.....	F ₄ corrugated selfed
122	21073P16.....	F ₄ corrugated selfed
23012	22012P3.....	<i>macrophylla</i> , pure line since 1907
098	22012P1 x 050P10.....	<i>macrophylla</i> x <i>sylvestris</i>
125	22121P2.....	F ₅ normal selfed
126	22121P2 x 050P5.....	F ₅ normal x <i>sylvestris</i>
127	22121P8.....	F ₅ normal selfed
128	22121P8 x 050P9.....	F ₅ normal x <i>sylvestris</i>
129	22121P1.....	F ₅ corrugated selfed
130	22121P1 x 050P10.....	F ₅ corrugated x <i>sylvestris</i>
131	22121P3.....	F ₅ corrugated selfed
132	22121P3 x 050P10.....	F ₅ corrugated x <i>sylvestris</i>
133	22121P4.....	F ₅ corrugated selfed
134	22121P4 x 050P10.....	F ₅ corrugated x <i>sylvestris</i>
135	22121P5.....	F ₅ corrugated selfed
136	22121P5 x 050P10.....	F ₅ corrugated x <i>sylvestris</i>
24140	23077P35 x 131P16.....	white <i>tabacum</i> x F ₆ corrugated
141	23077P35 x 131P35.....	white <i>tabacum</i> x F ₆ corrugated
142	23077P37 x 131P7.....	white <i>tabacum</i> x F ₆ corrugated
143	23077P37 x 131P11.....	white <i>tabacum</i> x F ₆ corrugated
144	23077P37 x 131P14.....	white <i>tabacum</i> x F ₆ corrugated
167	23131P11.....	F ₆ corrugated selfed
168	23131P18 x P8.....	F ₆ normal x corrugated

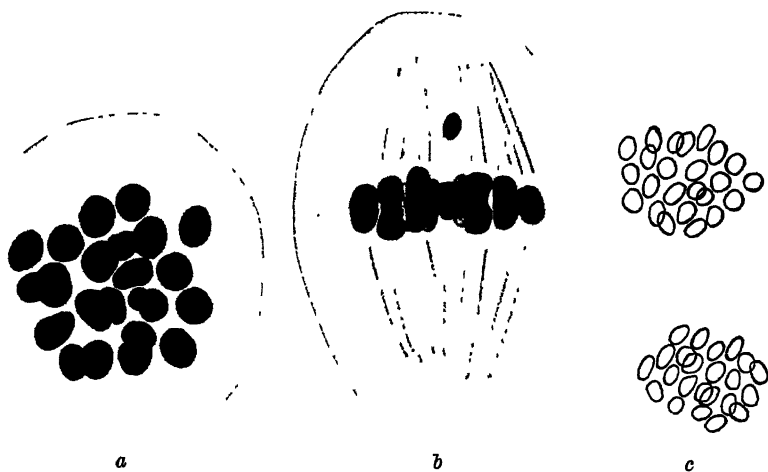


Fig. 3. *a*, Heterotypic metaphase of corrugated in polar view. The 23 bivalents and the single univalent are shown. *b*, side view of heterotypic metaphase of corrugated showing the single univalent characteristically placed off the equatorial plate. *c*, homotypic metaphases, 23 chromosomes in one plate and 24 in the other.

THE GENETIC BEHAVIOR OF CORRUGATED

On self-fertilization corrugated yields progenies consisting of approximately 60 per cent of corrugated and 40 per cent of normal plants. The normal segregants are identical with the original *macrophylla* parent. They are fully fertile and breed true when selfed. In table 3 the results of enumeration of 11 progenies of selfed corru-

TABLE 3
RESULTS FOR THE SELF-FERTILIZATION OF CORRUGATED PLANTS

Garden number	Generation	Total	Corrugated	Per cent
21073	F ₄	28	18	64.3
079*	F ₄	50	35	70.0
22092	F ₅	49	32	65.3
121	F ₅	47	26	55.3
122	F ₅	48	20	41.7
23129	F ₆	49	32	65.3
131	F ₆	50	27	54.0
133	F ₆	50	32	64.0
135	F ₆	50	35	70.0
137	F ₆	49	28	57.1
24167	F ₇	49	33	67.3
Total		519	318	61.3

* One white-flowering plant among the corrugated segregants.

TABLE 4

COMPARISON OF COROLLA TUBE LENGTH IN *macrophylla* WITH THAT OF CORRUGATED AND NORMAL IN DERIVATIVE LINESAll populations except 23012 represent F₂ progenies grown from sister F₁ plants.

Garden number	Parentage	Classes	Tube length in millimeters																Total	Mean tube length
			37	38	39	40	41	42	43	44	45	46	47	48	49	50				
23012	<i>macrophylla</i> pure line.....	normal.....	4	5	7	10	14	7	2	1	50	43.18±0.16		
125	normal selfed.....	normal.....	1	5	6	8	13	8	9	50	43.74±0.16		
127	normal selfed.....	normal.....	2*	1*	2	3	8	12	13	8	1	50	44.26±0.13		
129	corrugated selfed.....	normal.....	1	1	3	5	5	2	17	44.06±0.21		
		corrugated.....	1	2	7	11	6	5	...	32	47.06±0.15		
131	corrugated selfed.....	normal.....	1	6	13	3	23	44.78±0.10		
		corrugated.....	1	1	18	7	...	27	48.15±0.08		
133	corrugated selfed.....	normal.....	2	7	8	1*	...	18	44.35±0.11		
		corrugated.....	21	8	3	32	48.44±0.08		
135	corrugated selfed.....	normal.....	1	10	2	2	15	43.33±0.14		
		corrugated.....	5	26	4	...	35	47.97±0.06		
137	corrugated selfed.....	normal.....	2	8	10	1	21	43.48±0.11		
		corrugated.....	2	14	8	4	28	48.50±0.10		
Totals of populations segregating for normal and corrugated.....			1	4	24	30	29	5	93	44.04±0.07		
			1	2	8	19	85	32	7	154	48.01±0.05		

* Not included in computing mean tube length.

gated plants are set forth. Generations are counted from the back-cross, which was F_1 according to this system. In F_2 and F_3 other types than corrugated and normal appear as segregation products. Beginning with F_4 however, segregation for these two classes has been perfectly sharp and definite. During the same years eight parallel progenies from selfed normal segregants were grown. They contained 394 plants, all of which were normal. The interpretation of these results necessitated separate studies of ovule and pollen transmission ratios, which are reported below.

As we stated above, corrugated plants differ from normal ones in a variety of ways. In particular they exhibit a distinct increase in flower size, as may be seen by reference to table 4, which contains frequency distributions for tube length of corolla in populations grown in 1923. Those populations which consist of normal plants exclusively differ very slightly in mean tube length from *macrophylla*. In those which segregate for normal and corrugated, the tube length of normal plants differs very slightly from that of the normal populations. The tube length of corrugated plants, however, as shown by the last line in table 4, averages $3.97 \pm .14$ mm. longer than that of normal plants in the same populations.

A few exceptional measurements should be noted. Thus there are three plants in 23127 which clearly lie below the normal range. These have been tested and found to represent spontaneous production of another monosomic, fluted (cf. Clausen and Goodspeed, 1926). In 23133 one plant is classified as normal which had a flower measurement typical of the corrugated class. Although this plant has been tested we have been unable to determine its significance.

CORRUGATED \times SYLVESTRIS

When normal derivatives are crossed with *sylvestris*, the F_1 is identical in every respect with the original *macrophylla-sylvestris* hybrid. When corrugated is crossed with *sylvestris*, F_1 consists of two distinct classes; one red-flowering and identical with the normal *macrophylla-sylvestris* hybrid, the other white-flowering and exhibiting other distinctive morphological features. Both classes appear in reciprocal crosses, although probably in different proportions, as shown in table 5. This result demonstrates clearly that corrugated produces two kinds of gametes, and that homozygous corrugated

would be expected to be white-flowering, were it to be produced. In this connection it should be noted that a single white-flowering plant did appear in 21079, table 3, as a result of self-fertilization of corrugated. It was a very weak dwarf and totally sterile, so that it could not be followed further. Its appearance indicates that under certain conditions white-flowering plants may appear as rare segregants from corrugated.

TABLE 5
RESULTS OF CROSSING CORRUGATED WITH *sylvestris*

Type of cross	Garden number	Red	White	Total	Per cent white
<i>sylvestris</i> ♀ x corrugated ♂.....	20050	19	6	25	24.0
	22093	18	7	25	28.0
Total		37	13	50	26.0
corrugated ♀ x <i>sylvestris</i> ♂.....	21074	26	24	50	48.0
	080	32	18	50	36.0
	22095	25	23	48	47.9
	23130	36	13	49	26.5
	132	28	21	49	42.9
	134	33	17	50	34.0
	136	27	23	50	46.0
Total		207	139	346	40.2

The members of the red-and white-flowering classes differ in a number of particulars besides flower color. The white-flowering plants reach maturity later than red-flowering plants in the same populations, they are on the average about a foot and a half shorter, and they exhibit other rather indistinct differences in habit which, taken together, indicate a much closer resemblance to *sylvestris* than that of normal F_1 . The corolla tube length for white-flowering plants is also distinctly greater than that for red-flowering plants as is shown by the distributions in table 6. The average difference in this respect as shown by the last two lines of the table amounts to $8.87 \pm .09$ mm.

While the plants of these crosses of corrugated with *sylvestris* fall into two definite classes, based on flower color, there is some evidence from the tube measurements included in table 6 of production of occasional plants which differ in other respects from members of their flower color class. Thus in 23132 there was one white-flowering plant which had a tube length characteristic of the red-flowering class, and another plant which presented the reverse condition. Even in the

TABLE 6

COMPARISON OF COROLLA TUBE LENGTH IN THE NORMAL, RED-FLOWERING F₁, *macrophylla-sylvestris* HYBRID WITH THAT OF F₁ PROGENIES OBTAINED BY CROSSING NORMAL AND CORRUGATED DERIVATIVES WITH *sylvestris*

Garden number	Female parent (♂ = <i>sylvestris</i>)	Classes	Tube length in millimeters																								Total	Mean tube length
			40	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66								
23098	<i>macrophylla</i>	red	11	17	19	3	50	56.28±0.08						
126	normal	red	1†	2	21	16	7	3	50	55.76±0.09						
128	normal	red	3	9	8	6	2	28	54.82±0.14						
130	corrugated	red	2	16	10	8	36	54.67±0.10						
		white	3	4	5	1	13	63.31±0.17						
132*	corrugated	red	6	12	8	1	29	55.15±0.10						
		white	1†	5	11	2	2	...	21	64.05±0.13						
134	corrugated	red	33	55.56±0.09						
		white	1†	3	11	15	3	2	8	6	1	17	64.35±0.09						
136	corrugated	red	1	14	19	6	...	1	42	54.83±0.09						
		white	1†	8	63.86±0.21						
138	corrugated	red	1†	...	1†	1	12	10	2	27	54.52±0.09						
		white	1	1	1	8	9	1	23	63.48±0.18						
Totals of populations segregating for red and white ...			red	4	51	62	39	4	1	1	1	4	22	35	12	5	161	54.94±0.05		
			white	80	63.81±0.08		

* 23132 contained one red-flowering haploid *tabacum* plant with a tube length of 39 mm.

† Not included in computing mean tube length.

populations of normal \times *sylvestris* there are occasional off-type plants which present some difficulty in the way of interpretation. Thus in 23126 there is one plant with a short tube length, and there was another with shorter styles and filaments than is normal. The other population from normal \times *sylvestris* was uniform. In 23132, in addition to the two exceptions noted, there was one haploid plant, which has been described in another paper (cf. Clausen and Mann, 1924). In 23134 there was one red-flowering plant which bore exceptionally small flowers. In addition it was slightly less vigorous than the typical red-flowering plants, as shown by its more slender branches. In 23136 three exceptional plants were noted: P₈ was like the short-tubed plant in 23134; P27 exhibited short style length, associated with less robust type of growth and a narrower spread of corolla; and P48, a white-flowering plant, had rather short flowers for its type and its vegetative characters were somewhat more like those of red-flowering plants, although not in exact agreement. In 23138, P3 and P43 were duplicates of 23136P48; and P10 more nearly resembled normal plants of the selfed corrugated. Possibly this last plant was a stray, but the others cannot be accounted for in this way. Altogether there were 9 exceptional plants among 250, or 3.6 per cent.

CORRUGATED \times WHITE TABACUM

Since corrugated \times *sylvestris* segregates in F₁ into red and white, it seemed desirable to determine the relation of corrugated to white-flowering *tabacum* varieties. For this purpose two F₄ corrugated plants were crossed with 015, a white-flowering *tabacum* variety. The results from these crosses, together with results from parallel crosses of normal segregants with the white *tabacum* are contained in table 7. Flower measurements disclosed a similar but less marked difference between red- and white-flowering classes as we have noted for the parallel classes in the corrugated-*sylvestris* cross. These results indicate that the chromosome set involved in the production of corrugated is the one which bears the factors *W* - *w* for colored vs. white flowers in *tabacum*.

Two white-flowering plants appeared in 21078 from a cross of normal \times white *tabacum*, where only red-flowering plants were expected. They were of the same type as the white-flowering plants in the sister populations, 21075 and 21081, and they were probably

due to experimental error in transferring plants from seed pans to flats or from flats to the field. The same normal plant crossed with *sylvestris* gave a progeny, 21077, of 49 red-flowering and no white-flowering plants.

TABLE 7
RESULTS OF CROSSING CORRUGATED AND NORMAL WITH WHITE *tabacum*

Type of cross	Garden number	Colored	White	Total	Per cent white
Normal ♀ × white <i>tabacum</i> ♂.	21072	50	—	50	0.0
	078	48	2*	50	0.0
Total.....		98	2	100	0.0
corrugated ♀ × white <i>tabacum</i> ♂	21075	13	37	50	74.0
	081	18	32	50	64.0
Total.....		31	69	100	69.0
white <i>tabacum</i> ♀ × corrugated ♂	24140	47	3	50	6.0
	141	47	3	50	6.0
	142	50	0	50	0.0
	143	47	3	50	6.0
	144	49	1	50	2.0
Total.....		240	10	250	4.0

* The two white-flowering plants in 21078 were probably strays. In 21077 the same female parent crossed with *sylvestris* gave 49 red-flowering plants and no whites.

In 1924 five populations of white *tabacum* × corrugated were grown in order to determine the ratio of transmission of the corrugated complex through the pollen grains. The results of these crosses are also included in table 7. Since only 4.0 per cent of the plants were white-flowering, the transmission of the corrugated complex is evidently limited almost entirely to the ovules. This conclusion is also supported by the results of a cross of normal × corrugated, 24168, which produced 50 normal and no corrugated plants, which if included with the preceding data, reduces the percentage of pollen transmission to 3.3 per cent. These results are not, however, in agreement with those from cultures 20050 and 22093 recorded in table 5, which show 26.0 per cent of white-flowering plants; but possibly the data from crosses of corrugated × *sylvestris* are not comparable with those of corrugated × white *tabacum*. More complete data are evidently needed in order to determine the meaning of these and other discrepancies.

DISCUSSION

Since cytological examination has shown corrugated to be a monosomic, it is appropriate to compare its genetic features with those of the monosomic "fluted" described in the preceding number in this series. Unfortunately, however, much of the data collected for corrugated has been derived from crosses with *sylvestris*, and until further evidence is available it is impossible to state whether these results are comparable to those of *tabacum* crosses. From the results which have been obtained from *tabacum* crosses, however, the following comparisons of corrugated and fluted may be drawn:

Parentage	Fluted per cent	Corrugated per cent
Monosomic ♀ × normal ♂	59 5	69 0
Normal ♀ × monosomic ♂	2 8	3 3
Monosomic × self	42 6	61 3

Considering the extent of the data the behavior of the two forms is evidently closely parallel. Both show an excess of transmission through the ovules and a low ratio of pollen transmission. The most marked difference is in the ratio of transmission on selfing which in fluted is distinctly below expectation, based on ovule and pollen ratios. If we may be permitted to generalize from these results, it would appear that ovule transmission distinctly above the expected 50 per cent and a very low ratio of transmission in pollen grains are characteristic of monosomics in *tabacum*.

The chromosome present in the monosomic condition in corrugated is definitely shown to bear the factor W, for colored flowers, from the results of crosses with white *tabacum*. Presumably this chromosome, let us call it C, is one of the univalents in the F₁ *sylvestris*-*tabacum* hybrid, and the production of corrugated has its origin in the union of a viable nullo-C gamete from the hybrid with a normal *tabacum* gamete, for the original ancestor from which the corrugated race descended was shown by crossing with *sylvestris* to be haplo-C. The factors which are borne in this chromosome, therefore, probably have no parallels in the *sylvestris* complex. Attention is called to this fact as an explanation of the difficulty we have met with in transferring the color of *tabacum* to *sylvestris*. Such attempts have failed despite numerous and careful efforts to select partially fertile colored segregants in the backcross to *sylvestris*. Since this result would necessitate

not merely an exchange of a *sylvestris* chromosome for its *tabacum* homologue, but actual addition of the C-chromosome of *tabacum* to the *sylvestris* complex, the difficulties attending production of such a form are obvious. We do not know, however, that this is the only addition to the *sylvestris* complex necessary for the purpose; but even such a one may be expected to change materially other features of the plant, so that production of a *sylvestris* with colored flowers but otherwise identical with the species by segregation from the *sylvestris-tabacum* hybrid appears to be impossible.

The results obtained both from fluted and corrugated are pertinent to the problem of the return of *tabacum* derivatives to the parental type. It may be assumed that in the progeny of the original backcross of F_1 to *tabacum* most, if not all, of the plants will contain one or more unpaired *tabacum* chromosomes. If as with fluted and corrugated, pollen grains containing incomplete chromosome sets are largely eliminated in competition with those which have complete sets, then obviously a very rapid return to the parental condition would occur. It is therefore unnecessary to assume for these univalent chromosomes that production of the corresponding nullosomic condition would have a lethal effect, because such forms would be produced so rarely. Nevertheless it is extremely likely that they are comparatively low in viability and fertility, so that if produced they would still tend to be eliminated. Such at least was the case with the single white-flowering segregant obtained by self-fertilization of corrugated (cf. p. 94), but unfortunately the constitution of this plant was not determined.

It is unnecessary to enlarge upon the usefulness of monosomics in the Mendelian analysis, since this matter has been considered in the preceding paper in the series. It is, however, of interest to note that such forms may be obtained as segregation products from partially fertile interspecific hybrids of unbalanced chromosomal constitution. By taking advantage of this fact, it should be possible to obtain them more easily than by waiting for their spontaneous appearance.

SUMMARY

1. By self-fertilizing a partially fertile individual from the back-cross of the F_1 *macrophylla-sylvestris* hybrid to *macrophylla* an inconstant derivative called "corrugated" was established.

2. The character differences between corrugated and normal (*macrophylla*) are complex, but they behave as a unit in heredity.

3. Cytological examination shows that corrugated is a monosomic ($2n-1$) *tabacum* derivative.

4. Corrugated selfed produces corrugated and normal, both classes red-flowering, in proportion of approximately 61.3 and 38.7 per cent, respectively.

5. Corrugated ♀ \times *sylvestris* ♂ gives a dimorphic F_1 consisting of red-flowering plants identical with the normal F_1 *macrophylla-sylvestris* hybrid, and white-flowering plants of a different morphological type in proportions of approximately 59.8 and 40.2 per cent, respectively. In the reciprocal cross the proportions are approximately 74.0 and 26.0 per cent, respectively.

6. Corrugated ♀ \times white *tabacum* gives a dimorphic F_1 consisting of red- and white-flowering plants in proportions of approximately 31.0 and 69.0 per cent, respectively. In the reciprocal cross the proportions are approximately 96.7 and 3.3 per cent, respectively. These transmission ratios are approximately the same as those obtained for the monosomic, fluted.

7. The results of crosses of corrugated with white *tabacum* show that the chromosome set involved in the production of corrugated bears the factors, $W-w$, for colored vs. white flowers.

8. Applied to the problem of the return of *tabacum* derivatives from the *sylvestris-tabacum* hybrid to the pure parental condition, these results indicate that selective functioning of pollen grains is largely responsible for doubling-up of unpaired *tabacum* chromosomes.

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INTERSPECIFIC HYBRIDIZATION IN
NICOTIANA. IV

SOME CYTOLOGICAL FEATURES OF THE
PANICULATA-RUSTICA HYBRID AND ITS
DERIVATIVES

BY

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INTERSPECIFIC HYBRIDIZATION IN NICOTIANA. IV

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INTRODUCTION

The large number of interspecific hybrids which we have obtained in *Nicotiana* provide exceptional material for a study of cytological phenomena of a variety of sorts. A number of these hybrids have been examined in a preliminary way and so many points of cytological interest appear that we are subjecting as many of them as possible to critical cytological examination. Particular attention is at present being given to 12 x 24 contrasts, of which 12 have been thus far secured.

Of this group *sylvestris-tabacum* and *paniculata-rustica* are especially interesting because of the partial fertility they exhibit. Some cytological data have already been published on the former (Goodspeed, 1923) and another report dealing with derivatives of this hybrid will shortly be published. The present paper deals primarily with cytological phenomena in the *paniculata-rustica* hybrid and its derivatives. This hybrid is of particular interest because as East (1921) has shown, stable *rustica* derivatives differing from the original *rustica* parent may be obtained from it.

Previous investigations have been summarized by East, so that only a few statements by way of orientation are necessary here. *Paniculata* is generally recognized as a peculiarly well defined and stable species. *Rustica* on the other hand is highly polymorphic and exists in a large number of well defined varieties; such as *scabra*, *texana*, *brasiliä*, *humilis*, *pumila*, etc. Photographs and descriptions of these species and varieties are given in Setchell's (1912) account of the genus and in East's (1921) report of genetic investigations on the hybrid. This hybrid has been a frequent subject of investigation since the appearance of Kölreuter's 1763 report; but East's studies represent the only attempt known to us to interpret the results in terms of modern

genetic theory. Unfortunately, however, his interpretation suffers by reason of a failure to recognize the difference in chromosome number in the two species; consequently it has seemed appropriate to determine, in so far as is possible, the relation of this situation to the genetic results. The present report is preliminary to an attempt to determine the nature of the recombination products secured from these two species.

PANICULATA, RUSTICA, AND THE F₁ HYBRID

During the past six seasons we have grown a number of cultures of the F₁ *paniculata-rustica* hybrid, employing a number of varieties of *rustica*; in particular *brasilia*, *pumila*, and *scabra*. The results have been identical with those reported by East. Hybridization is readily accomplished, particularly when *rustica* is used as the female parent. The F₁ hybrid is exceedingly uniform, vigorous, and prevaillingly of *rustica* characteristics. Practically all the data reported in this paper were obtained from the *brasilia-paniculata* hybrid. As may be recalled, *brasilia* is a unique *rustica* variety, characterized by its stout, robust habit, large, bullated leaves, and late maturity, features which in our experience are recessive in crosses with the more typical, slender *rustica* varieties. While vigorous, the F₁ hybrid is of the slender type of other *paniculata-rustica* hybrids; so that the special features characteristic of *brasilia*, which are recessive in intervarietal *rustica* hybrids, also are recessive in the interspecific hybrid. If the comparison is made of the hybrid with the immediate parents in this instance one might conclude that it exhibited a strong *paniculata* influence; but as a matter of fact, compared with more typical *rustica* varieties this apparent effect is seen to depend wholly upon suppression of the *gigas* features of *brasilia*. The degree of fertility exhibited by the hybrid in our cultures was comparatively low. Free-blooming plants in the field were observed to cast their flowers without setting seed. Backcrosses with the hybrid as female parent to *paniculata* and *rustica* always led to retention of the capsules and production of some seeds; but self- and sib-pollinations gave no seeds. Later, however, under greenhouse conditions seed was also obtained from such pollinations.

As previously reported (Goodspeed, 1923), the correct haploid chromosome numbers for the parents of this hybrid are *paniculata* 12, *rustica* 24, and not 24 pairs in both as stated by East. All the varieties and forms of *rustica* which we have grown, ranging from the dwarf variety, *pumila*, to the comparatively gigantic *brasilia*, are $n=24$. The somatic number in the F₁ and the cytological phenomena at its meiotic

divisions confirm these parental counts. The situation as to chromosome numbers is illustrated in figure 1, where typical first metaphases in polar view of *paniculata*, of *rustica* var. *brasilia*, and of the F_1 are shown. These and following text figures were drawn exclusively from acetocarmine preparations of P.M.C., in some cases from cells intact and in other cases from crushed cells. As a result, chromosome sizes and cell topography as shown are somewhat variable. There is, of course, no reason why a certain proportion of the difference in chromosome size shown may not be actual and a reflection of size differences in the parental haploid sets; but lacking duplicate and controlled fixations we prefer to assign these differences to technique.

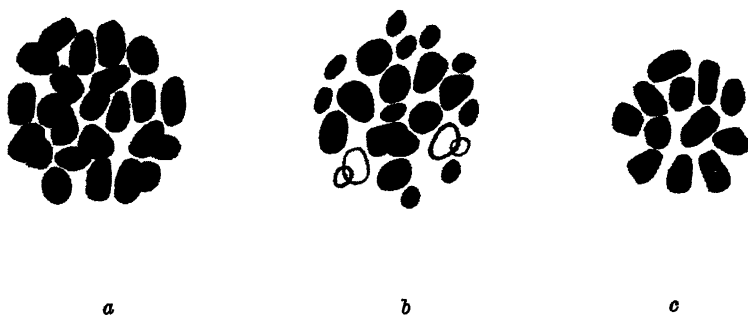


Fig. 1. Heterotypic metaphases, polar view. * *a*, *Nicotiana rustica*;
b, F_1 *rustica* - *paniculata*; c, *Nicotiana paniculata*.

In *paniculata* and in *rustica* the diakinesis is well formed, in the one case 12 and in the other case 24 pairs of chromosomes are uniformly to be counted. In the F_1 , on the other hand, the presence in diakinesis of univalents as well as bivalents is clear and it is often possible to count 12 of each type. The pairs are always well formed and the distinction between paired and unpaired chromosomes is sharp. This distinction is clearly reflected at first metaphase where, as shown in figure 1b, 12 large and 12 small chromosomes appear. As contrasted with F_1 *sylvestris-tabacum* (cf. Goodspeed, 1923) and with many other species hybrids in *Nicotiana* which we have seen, the distinction between all members of the two chromosome classes is always striking. Just as in many hybrids exhibiting the *Drosera* scheme, the bivalents at first metaphase are well concentrated in an equatorial plate, with the singles lying at random above or below it. Similarly, the bivalent partners are distributed in an orderly manner to the poles and the univalents tend to occupy the equator following the disjunction of the bivalents

and then undergo some type of distribution. These points are illustrated in figure 2, *a, b*.

As will be seen in figure 2*b*, the univalents appear to be undergoing division. Similar pictures were given by large numbers of late first anaphases which were examined. Counts at second metaphase, however, indicate that division of the univalents in first anaphase is not accomplished (fig. 2*c*). The following summary of our data on this

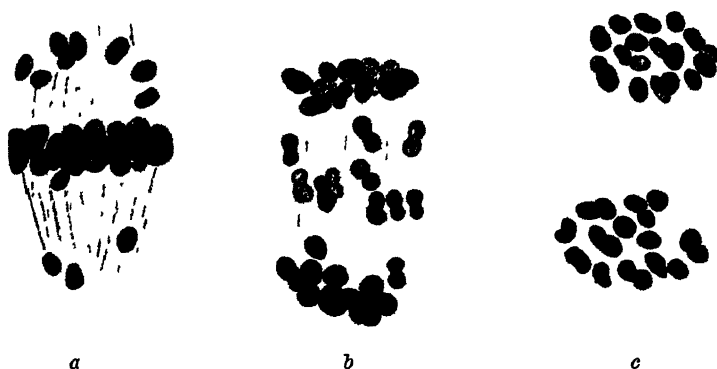


Fig. 2. *F₁ rustica-paniculata*—25143; *a*, heterotypic metaphase, side view, showing 12 bivalents in an equatorial plate and 12 univalents off the plate; *b*, heterotypic anaphase, bivalent partners at the poles and univalents apparently dividing; *c*, homotypic metaphase plates in polar view, 18 chromosomes in each.

point indicates that the sum of the homotypic chromosomes was never more than 36; and that typically random distribution of univalents occurs in the first division:

	12	13	14	15	16	17	18
Type of distribution	24	23	22	21	20	19	18
Binomial coefficients $(a+b)^{18}$	1	12	66	220	495	792	462
Number of counts	—	5	3	4	12	13	28
Calculated	0.0	0.4	2.1	7.0	15.7	25.1	14.7

We do not contend that division at first anaphase of one or of a few of the univalents never takes place. Indeed the appearance of the univalents at that stage gives every reason to suppose that division sometimes must be accomplished, but simply that it is not the rule in the *paniculata-rustica* hybrid. We would call attention, in this general connection, to the danger of drawing final conclusions in similar hybrid material from the appearance of univalent chromosomes at first anaphase. In our experience and also to judge by published figures

of second metaphases of P.M.C. of other hybrids, paraffin sections, after fixation and staining according to conventional methods, are likely to provide unreliable pictures of meiotic stages later than first telophase. Certainly in species hybrids of *Nicotiana*, paraffin sections may show prophases and heterotypic phases excellently fixed but homotypic stages in a condition suggesting considerable irregularity and even breakdown of chromatin with the result that chromosome counts are almost entirely out of the question. Absolutely equivalent material prepared according to the aceto-carmin method gives clear pictures of distinct and well separated homotypic chromosomes which often can be counted with great accuracy.

Only very rarely are chromosomes seen in the plasma at first telophase, although at times one or two chromosomes appear to be slightly off the homotypic metaphase plates. The second division is, on the whole, rather normally accomplished. A few chromosomes often lag at late second anaphase but in most cases they appear to complete their division or are included undivided in the four resulting nuclei. Early telophases like the one shown in figure 3 occur infrequently and even in such cases it is not clear that the chromosomes in the

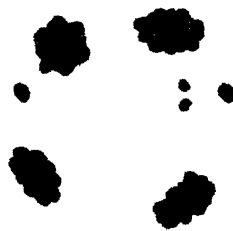


Fig. 3. *F₁ rustica - paniculata*.
Early homotypic telophase,
showing lagging chromosomes.

plasma do not ultimately come to be included in the granddaughter nuclei. An examination of tetrads confirms this statement in that few contain microcytes or "micronuclei" (cf. Clausen and Goodspeed, 1926).

Of 100 tetrad stages examined, all showed four cells and in only four cases was a single small microcyte found.

Summing up meiotic behavior in P.M.C. of the *F₁*, we would emphasize that (1) there are 12 bivalents and 12 univalents at the heterotypic metaphase; (2) the bivalent partners are regularly distributed to the two poles; (3) there is a simulated division of the univalents, but the sum of the chromosomes at the homotypic is rarely, if ever, more than 36; (4) the homotypic division is passed through rather normally.

Unfortunately, material has not been available for a thorough study of E.M.C. Such prophases and heterotypic stages as we have seen exhibit behavior corresponding to that described above for P.M.C.

PANICULATA DERIVATIVES¹

A plant of the *F*₁ *brasilia-paniculata* hybrid, 24181 Pe, was backcrossed as a female parent to *paniculata*, yielding about 188 well formed seeds in 15 capsules. Of these, 167 seeds, the product of 12 capsules, when sown in 1925 under the garden number 25144, produced only 44 plants which survived up to the time of transfer to the garden. The plants thus secured were highly diverse, so much so that no method of classification appeared to be feasible. They varied in robustness from plants which grew only six or eight inches high to others which attained a height of three and a half to four feet. Extreme variation was also exhibited in flower size, leaf size and shape, habit of growth, and in fertility. Some of the plants produced misshapen flowers, a few cast their buds at a very early stage, so that it was impossible to work with them. Ten of what appeared to be the most fertile plants were again selected for backcrossing to *paniculata*; but only three of these produced any seed at all, although parallel backcrosses of the *F*₁ hybrid made at the same time under identical conditions always produced seed. Of the three successful backcrosses, one yielded an abundance of seed and the other two, a scanty amount comparable to that of *F*₁. In so far as our experience is concerned, fertility in this population appeared to average lower than in *F*₁.

The chromosome situation at the heterotypic metaphase was determined individually for 34 plants. All plants clearly exhibited twelve pairs or bivalents plus a variable number of univalents, as shown in the following enumeration:

Number of univalents.....	0	1	2	3	4	5	6	7	8	9	10	11	12
Number of plants....			1	1	4		8		4	2			14*
Calculated.....	0	0.05	0.3	1.1	2.4	3.9	4.5	3.9	2.4	1.1	0.3	0.05	0.0

* Omitted from consideration in calculations.

Heterotypic chromosome combinations of five plants are shown in figure 4, primarily to indicate the sharp distinction between bivalent and univalent chromosomes. The predominance of 12_{II}+12_I combinations shown by the original counts gave rise to a suspicion that an abnormally early division of univalents was occurring in some cases.

¹ Plants obtained by backcrossing the *F*₁ hybrid to *paniculata* are here designated as *paniculata* derivatives.

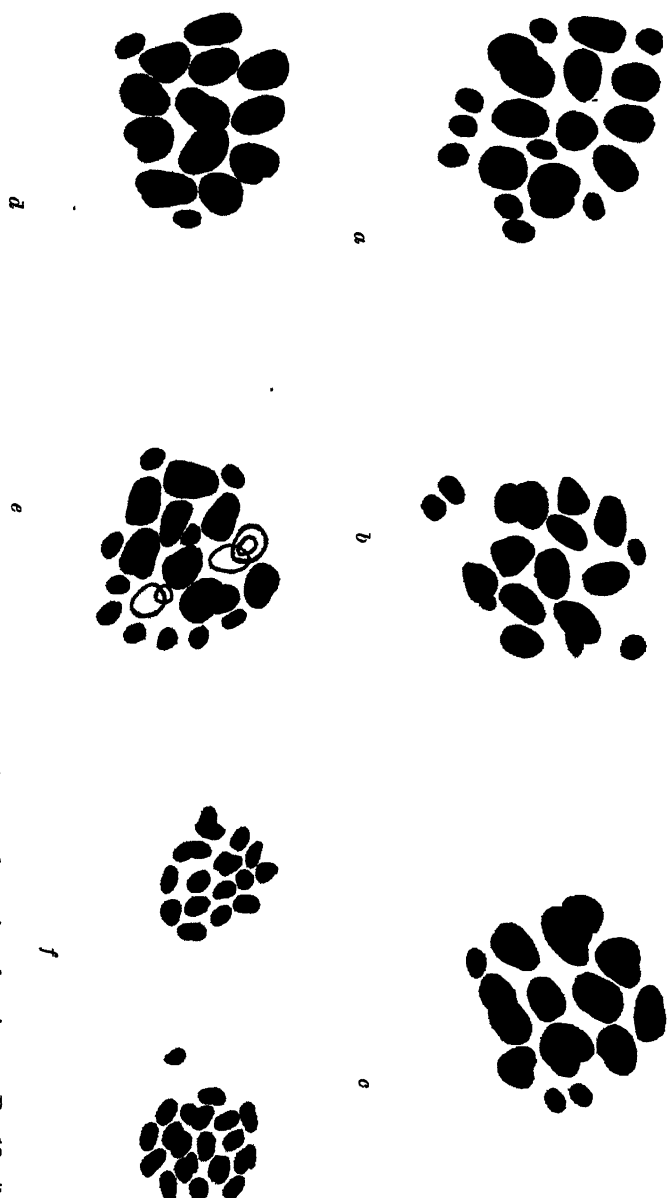


Fig. 4. *F₁* backcross (*rustica* - *paniculata*) - *paniculata* - 25144; a to e, heterotypic metaphases in polar view; a, $P_{81}, 12_{11} + 9_i$; b, $P_{81}, 12_{11} + 6_i$; c, $P_{41}, 12_{11} + 3_i$; d, $P_{81}, 12_{11} + 2_i$; e, $P_{81}, 12_{11} + 12_i$; f, homotypic metaphases of $P_{81}, 18$ chromosomes in right hand plate, 21 in left hand plate, and 1 chromosome in plasma.

Although our previous experience gave no ground for such an explanation of the situation, we gave some attention to this point. Study of diakinesis and chromosome number at homotypic was, however, convincing as to the correctness of the first counts. Figure 4f is introduced in part to give evidence in this connection. It shows homotypic metaphases of 25144P6, the heterotypic metaphase of which is shown in figure 4e. There were 12 bivalents and 12 univalents at the heterotypic and 18 chromosomes in one homotypic plate, 21 in the other, and one chromosome apparently in the plasma. On the assumption that the 12 univalents at the heterotypic metaphase actually were the product of six which had prematurely divided, the homotypic sum in this case cannot be explained without the assumption that certain univalents had divided twice in the first division. Such evidence is not so convincing, however, as that obtained at diakinesis, where $12_{II}+12_I$ could readily be counted, the pairs being well formed.

In general, the sequence of events in first anaphase of 25144 corresponds to that described for the true F_1 . The partners of the 12 bivalents were separated regularly to the poles, and the univalents, in the majority of cases undivided, were distributed at random. In other words, in $12_{II}+12_I$ heterotypic combinations the sum of the homotypic metaphases was usually 36. As contrasted with the true F_1 , there was, however, a considerable number of instances in which division of univalents at first anaphase (cf. fig. 4f) was found to have occurred.

In the case of plants whose chromosome garniture involved a small number of univalents there is evidence that they rarely divide in the first division but often remain in the plasma as a result of anaphase lagging. We have seen cases in which the homotypic showed two well-organized metaphase plates, each containing 12 chromosomes, the small number of univalents involved lying scattered about in the plasma. Some of them undoubtedly are included in the granddaughter nuclei but there appeared to be a general tendency toward a more or less complete loss of univalents in plants where their original number was small.

The above data appear to indicate that distribution of univalents is effected in two distinct ways: (a) at random, giving a series of gametes containing from 0 to 12 of the univalents in frequencies corresponding to the coefficients of the binomial $(a+b)^{12}$ and (b) in some way by which all of the univalents are included in the gamete. The chromosome numbers appearing in classes 2-9 in the above distribution seem to indicate random survival of combinations resulting from random distribution, a fact rather difficult to reconcile with the high degree of abortion exhibited in the pollen of the hybrid.

The presence of a high proportion of $12_{II}+12_I$ plants led to a careful comparison of this group among themselves, with other plants in the population, and with the F_1 , ten plants of which were growing in the garden under number 25143. They were not identical with F_1 , they were not uniform, and they exhibited no characteristic features as a group compared with plants having a smaller number of univalents. These facts preclude their origin by apogamy, and apparently necessitate occurrence of some form of distribution by which the bivalent partners are distributed at random and the univalent chromosomes either divide equationally or are comparatively frequently distributed as a group to one pole in the heterotypic division. In the absence of E.M.C. studies it is only possible to speculate as to the explanation of this phenomenon. Distribution of all univalents to one pole has been described both by Blackburn and Harrison (1921) and Täckholm (1922) as a regular phenomenon in certain forms of *Rosa*.

RUSTICA DERIVATIVES

The same F_1 *brasilia-paniculata* plant, 24181 Pe, from which the above described *paniculata* derivatives were secured, was also backcrossed as a female parent to *brasilia*, yielding as a result about 147 well formed seeds in 15 capsules. Of these, 95 seeds, the product of three capsules, when sown in 1925 under the garden number 25145, produced only 17 plants which survived up to the time of transfer to the field. These plants were also highly diverse; but in general they were vigorous individuals, prevailing *rustica* in type, but none of them closely resembled the original *brasilia* parent. The degree of fertility was comparatively high. Of twelve plants selfed in duplicate, three produced seed; two an abundance and one a fair quantity. Four plants backcrossed to *brasilia* all produced seed; two abundantly and two scantily. Fertility in these derivatives was obviously higher on the average than that of *paniculata* derivatives and F_1 .

Of 15 plants which came to maturity, the chromosome number of 13 was determined. The distinction between bivalents and univalents was, in many cases, quite sharp as indicated in figures 5 and 6a. In the case of two plants entirely reliable counts were not obtained. In these plants there was no doubt as to the total number of chromosomes on the heterotypic metaphase but in the case of 2 or 3 chromosomes a final decision could not be made. Of the 13 plants, 7 were $18_{II}+6_I$ and 4, $20_{II}+4_I$. The two doubtful plants were also of similar chromosomal constitution.

The homotypic division in 25145 was, in general, not so regular as in 25144. Figure 6,b,c, shows the occurrence of lagging chromosomes at both telophases.

These results agree with those secured from the *paniculata* derivatives with the exception that no 24_{II} group corresponding to the $12_{II}+12_I$ group of *paniculata* derivatives was secured, a discrepancy for which we have no explanation.

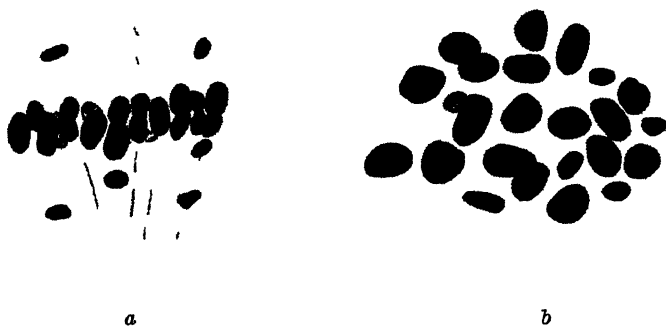


Fig. 5. F_1 backcross (*rustica* - *paniculata*) - *rustica* - 25145 a, P_8 heterotypic metaphase in side view, $18_{II}+6_I$; b, P_{13} , polar view, $18_{II}+6_I$.

GENETIC SIGNIFICANCE

In discussing the genetic significance of these phenomena it is necessary to draw largely on the results which East has reported for this hybrid. It will be recalled that his conclusions are based upon F_2 and subsequent generations obtained by self-fertilization, whereas we have employed the method of backcrossing F_1 to the parental species, which obviously simplifies the problem of cytological study. By self-fertilizing the F_1 *paniculata-rustica* hybrid East secured a highly varied assemblage of F_2 plants most of which bore a general resemblance to *rustica*, but a few were practically equivalent to *paniculata*. From the *rustica* group self-fertilization rapidly led to the establishment of constant derivatives, some of which were close approximations of existing *rustica* varieties. On the other hand, the *paniculata* group gave rise only to derivatives identical in every respect with *paniculata*. The salient feature of these investigations is the demonstration that, starting with a single variety of *rustica*, by hybridization with *paniculata* stable recombination products eventually may be obtained which duplicate the characteristic features of a wide range of existing *rustica* varieties.

Assuming that the constant derivatives of the *rustica* category which he secured had 24 pairs of chromosomes, a reasonable assumption in view of the fertility of their hybrids when crossed together and with *rustica* varieties, they must have resulted from replacement of certain *rustica* chromosomes with *paniculata* homologues. The fact that different kinds of *rustica* derivatives were secured may then be taken

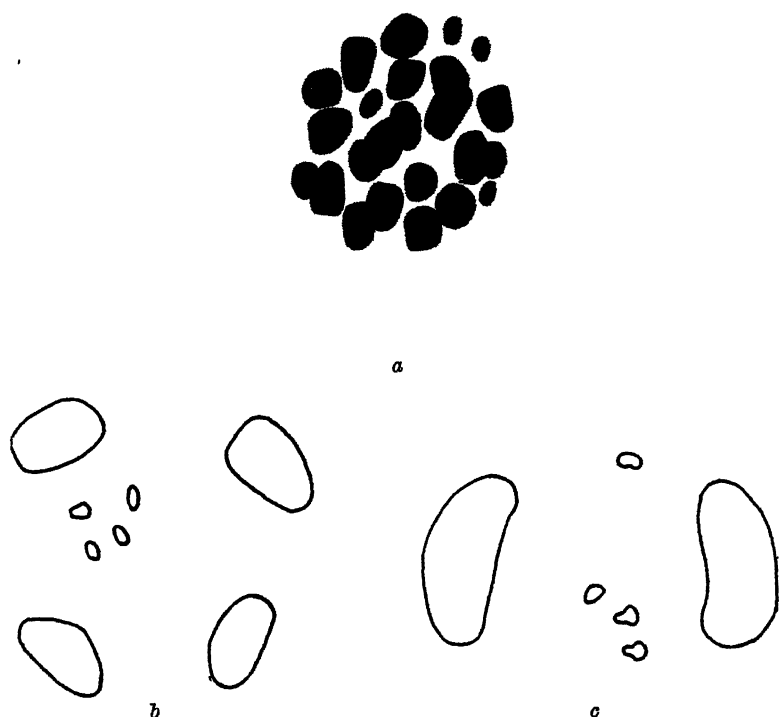


Fig. 6. 25145P₁₁. *a*, heterotypic metaphase, polar view, 20_n + 4_i; *b*, homotypic telophase to show lagging chromosomes; *c*, same at heterotypic telophase.

to indicate that homology of *paniculata* and *rustica* chromosomes does not connote identity of genetic content, for the differences among these derivatives are evidently due to the fact that the *paniculata* chromosomes bear some factors different from those of the *rustica* homologues.

The same conclusion is reached from an examination of the 12_n + 12_i group of plants in the backcross of the F₁ *paniculata-rustica* hybrid to *paniculata*. Presumably these plants have the same set of univalent chromosomes as the F₁ hybrid, therefore any differences which occurred

among them must be referred to the twelve pairs of bivalents. Now these plants were obviously different from the F_1 hybrid and they differed among themselves in size and habit of plant, size and shape of leaf, type of inflorescence, rate of growth, size and shape of flowers, etc., in fact no two of them looked enough alike to be considered identical genetically. These results indicate that some of the bivalents consisted of two *paniculata* members, instead of a *paniculata* and a *rustica* member as in F_1 , consequently again, substitution of a *paniculata* chromosome for a *rustica* homologue changes plant characters, and indicates that the two homologues differ in some genetic factors.

In view of the cytological conditions replacement of elements of *rustica* by *paniculata* is evidently restricted to the twelve *rustica* chromosomes which pair with *paniculata* homologues. East's results apparently show that such replacement is possible, as evidenced by a variety of diverse *rustica* derivatives. However, the corresponding reciprocal replacement of *paniculata* by *rustica* elements seems to be impossible, for no constant *paniculata* derivatives differing from the parental type were secured. It is hardly possible to conclude that such replacements might occur in *paniculata* without occasioning a detectable change in type, in view of the very distinct somatic effects obtained by replacement of *rustica* with *paniculata* chromosomes.

Evidently the time is rapidly approaching when this hybrid, already studied over a period of 160 years, may be subjected to a much more decisive analysis than has heretofore been possible. With present-day methods of investigation, it is perfectly feasible to replace *rustica* chromosomes one by one with *paniculata* homologues, and thereby to observe what somatic effect each replacement has and what cytological and genetic phenomena attend it. It should also be possible to make similar replacements in the *paniculata* complex and to add non-homologous *rustica* chromosomes to it. It seems possible that in this way a more accurate idea may be obtained as to the genetic relationships of these two species. We have already embarked upon this program, but it will be some time before final results will be secured.

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BY

T. H. GOODSPEED AND R. E. CLAUSEN

We have been successful in crossing *Bigelovii* with a number of other species of *Nicotiana*; with *suaveolens*, *glutinosa*, *nudicaulis*, and *Tabacum*. Some of these F_1 hybrids have been grown in the past, others were produced for the first time this last season. Since the cytological features of F_1 *Bigelovii* \times *suaveolens* and *Bigelovii* \times *glutinosa* appear to be identical, we have thought it desirable to describe and discuss them jointly.

The taxonomic status of the *Bigelovii* group has been discussed by Setchell (1912 and 1921). The type of *N. Bigelovii* proper, is a low spreading annual, with short internodes, ascending branches, rather large salverform white flowers, petioled leaves, and herbage glandular-pubescent and ill-smelling. Variety *Wallacei* was used as a parent in the hybrids under discussion. It differs morphologically from the type in its more slender erect habit, and in its flowers, which are smaller and possess a more slender tube. Both *suaveolens* and *glutinosa* have been described and figured by Setchell (1912). The former is a well recognized and stable species widely cultivated in botanical gardens and, to judge by these cultures which we have seen, subject to little variation. Much the same statement can be made in the case of *glutinosa*. These three species differ in chromosome number. In a previous report (Goodspeed, 1923) *Bigelovii* was placed in the group of species possessing 24_n , *glutinosa* in the 12_n group, and *suaveolens* was determined as 18_n . Further investigation confirms these counts for the first two of these species, but 16_n rather than 18_n is now recognized to be the correct count in the case of *suaveolens*.

We have had no difficulty in obtaining hybrids of *Bigelovii* and *suaveolens*. The cross and its reciprocal are identical and, as com-

pared with F_1 *Bigelovii* \times *glutinosa*, at least, incline rather strongly toward *suaveolens*. Their habit is reminiscent of *suaveolens* in uprightness and strength of the main axis which branches from the three or four uppermost nodes, followed by the production of flowering shoots from the rosette. The flower is white, the tube somewhat shorter than in *suaveolens*, and the diameter of the limb greater than in either parent. Except possibly on the calyx, there appear to be few if any glandular hairs, with which all vegetative organs of *Bigelovii* are densely clothed. The leaves incline in shape toward *Bigelovii*, the venation and color of which they possess. The *Bigelovii* \times *glutinosa* hybrid was produced for the first time this past season. Some seed

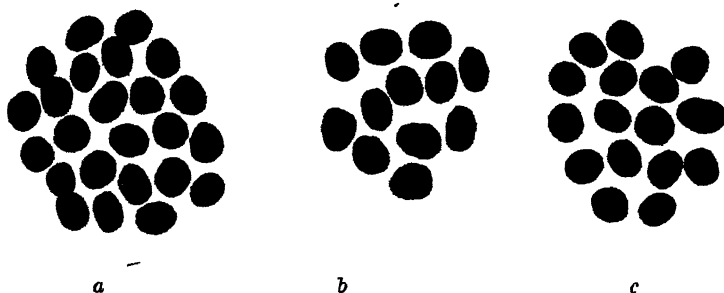


Fig. 1. P.M.C.—M I, polar views of a, *Nicotiana Bigelovii*; b, *N. glutinosa*; c, *N. suaveolens*.

of this hybrid and its reciprocal was obtained but we were able to bring to flower only two plants of *Bigelovii* \times *glutinosa* while the reciprocal failed in the cotyledon stage. These two plants are close to *Bigelovii* in external morphology. They possess its habit, leaf shape, color, and venation, although the petiole is a little more pronounced. All vegetative organs bear a heavy coating of glandular hairs. The influence of *glutinosa* is seen in the flowers, where the corolla limb shows a pinkish-violet tinge and a somewhat bilabiate tendency is apparent.

We have already published¹ (1923 and 1926) descriptions of the cytology of F_1 *sylvestris* (12_{II}) \times *Tabacum* (24_{II}) and F_1 *paniculata* (12_{II}) \times *rustica* (24_{II}) in which the main specifications of the so-called "Drosera scheme" were followed. In both the haploid chromosome set of the parent with the smaller number conjugated in the prophase of the meiotic divisions with an equal number of chromosomes from

the haploid set contributed by the other parent, the bivalents formed behaving in the usual fashion at A I and the univalents, undivided, being distributed by chance to the two poles. We are cognizant of similar chromosome behavior in others of our interspecific hybrids, and there also appear to be cases in which conjugation between members of the two haploid sets is not so precise. The two hybrids under discussion are, however, the first in which we have found a complete lack of any observable conjugation at meiosis.

The cytological evidence in the case of F_1 *Bigelovii* \times *suaveolens* is quite complete and is based upon an extended series of studies of both fixed and aceto-carminic material. In the case of F_1 *Bigelovii* \times *glutinosa* only aceto-carminic preparations have been examined, but the evidence as to chromosome behavior is quite complete. Figure 1 shows the parental chromosome sets, the three M I plates being drawn from aceto-carminic material.

In our experience, the post-synizesis stages in these as in other *Nicotiana* species are sharply defined and, in the case of diakinesis, third contraction, and M I, well sustained. The same is true of many of the hybrids between them, which consistently exhibit a greater or lesser degree of chromosome pairing. That is to say, there is no difficulty in these hybrids in obtaining aceto-carminic preparations of P.M.C. which show an abundance of countable diakineses and M I stages. On the other hand, in the hybrids which show an apparently complete lack of chromosome conjugation post-synizesis stages up to A I are, so far as we can judge, passed through with considerable rapidity. We commonly find an anther in which middle synizesis predominates accompanied by a few third contraction stages (fig. 2a), rarely a number of what appear to be late diakinesis (fig. 2b) and A I (figs. 3a and b). Another anther from the same bud will often contain all stages from A I to mature tetrads in approximately equal numbers, with a tendency to predominance of stages following the formation of the granddaughter nuclei. We hope at some future time to make a more critical study of sequences and time relations in pro-phases and distributional stages. Undoubtedly they are responsive to environmental influences and growing the plants under sub-optimum conditions might produce a retardation, especially of the prophase sequence, and permit further study of diakinesis and very early M I.

We have recently obtained interesting and apparently convincing evidence as to the relation of the multipolar spindle to the peculiarly

scattered condition of the chromosomes in M I and to their distribution to the poles. Figures 4*a, b* show the chromosomes in what appears certainly to be the multipolar spindle. In figure 4*a* at least three poles can be distinguished and we assume that distribution has begun to take place along the axis of the two major and opposite peaks, while the chromosomes near the third pole have either been drawn toward it or are caught in the "backwater" which it has created. This suggestion introduces the question of the time element (periodicity) in P.M.C., with which we are becoming somewhat impressed as a result of our studies of meiosis of interspecific hybrids. In the case under discussion



Fig. 2. *F₁ Bigelovii* × *suaveolens*, P.M.C.—*a*, third contraction, all chromosomes not shown; *b*, diakinesis, all 40 chromosomes unpaired.

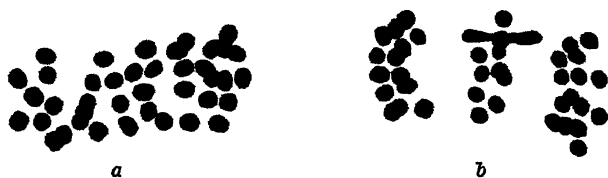


Fig. 3. P.M.C. *a*, *Bigelovii* × *suaveolens*, "normal" M I, side view; *b*, the same, A I, side view, to show apparently a division (?) in the case of one chromosome, observed only rarely.

it would appear that for any one P.M.C., at a certain definite period following synizesis, random distribution of the univalents must occur. Usually the spindle has by this period attained the mature bipolar condition and the A I has the appearance shown in figure 3*a*. The result of such a "normal" distributional mechanism leads to the production of daughter nuclei in most cases with approximately the same number of chromosomes in each, and only very rarely to cases in which one nucleus contains almost all of them.

On the other hand, when the bipolar condition has not been attained previous to the inception of this definitely fixed period, those chromosomes in the zone of the more bipolar portion of the spindle begin to

be distributed, the remaining chromosomes being held more or less passive near by. At the end of a definite distributional period, daughter nuclei are rounded up, with the result that the chromosomes which have not been distributed will, in such cases as the ones figured (fig. 4a, b), be included along with those which have been distributed to the nearby pole, producing a very considerable inequality in the number of chromosomes contained in the two daughter nuclei. In other words, the effect of such an "abnormal" distributional mechanism would be to produce a far too large class of cases in which the number of chromosomes at the two poles is very different.



Fig. 4. *Bigelovii* \times *glutinosa*, a, M I, distribution of univalents under way before the complete establishment of the bipolar spindle. The group in the circle under the influence of a third pole of the multipolar spindle; the 6 chromosomes in the arc and the ten uppermost chromosomes having been distributed; and the seven in the center being distributed. b, T I, it is assumed that 12 chromosomes will enter one daughter nucleus, the remainder of the 36 will be rounded up in the other daughter nucleus.

We have made some study of E.M.C. Figure 5a shows what we take to be A I and corresponds in general details to conditions illustrated for P.M.C. in figure 3b. The great attenuation and the curving of the spindle is quite characteristic for E.M.C. M I and does not appear to be due to fixation. Figure 5b represents a condition observed a number of times which we interpret as T I although it might perhaps be taken to mean that a premature and generally abnormal homotypic division is in progress.

Interkinesis in P.M.C. shows relatively few chromosomes left in the plasma as a result of A I lagging, at least when one considers that all

the chromosomes are apparently being distributed at random as univalents. In the majority of cases the two daughter nuclei are of approximately equal size but in a considerable number of cases the distinction is striking.

The situation at M II is interesting as well as convincing as to the A I evidence that univalents are being distributed at random in the first division both in what we have referred to as "normal" and as "abnormal" achromatic figures. In all P.M.C. where, at M II, the chromosomes could be counted with certainty, a total of 36 or 40 was observed. In many cases the two homotypic plates contained approximately equal numbers (fig. 6c) but in a considerable number of P.M.C. the number of chromosomes in one plate was greatly in excess of that in the other. This situation is illustrated in figures 6b and 8a. The fact that less than half the number of chromosomes contained in



Fig. 5. *F₁ Bigelovii* × *suaveolens*, E.M.C., a, M I, showing the long curved spindle; b, A I or possible M II.

the parental set characterized by the smaller number—in the hybrids under discussion, 12 or 16—is found rather often in one of the M II plates, is evidence in such hybrids that complete conjugation between or within parental haploid sets has not occurred. Indeed such evidence would suggest that no pairing at all had taken place and that the total chromosome complement of the hybrids was distributed as univalents at A I.

T II conditions are often highly irregular, as might be expected from the appearance of some of the M II stages. Even where the chromosomes are almost equally divided in two well organized M II plates, and there are no chromosomes left in the plasma after T I, chromosome lagging in A II results in the failure of inclusion in the granddaughter nuclei of a number of chromosomes. The situation at T II is illustrated in figures 7b and 8b and the resulting tetrads contain a great variety in size and number of major cells, microcytes, and micronuclei.

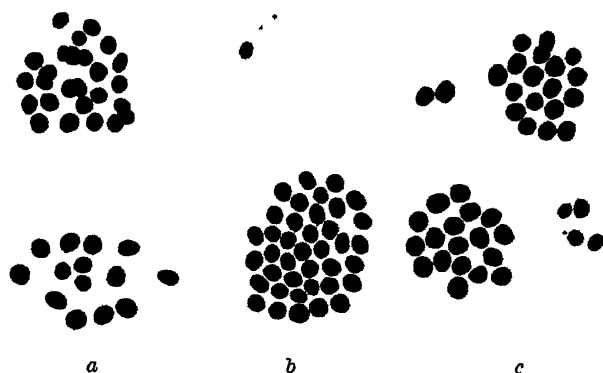


Fig. 6. *F*₁ *Bigelovii* × *suaveolens*, M II, P.M.C., *a*, 26 in one plate, 14 in the other; *b*, 39 in one, 1 in the other; *c*, 18 in both major plates, 2 at lower right dividing, 2 at upper left in the plasma.

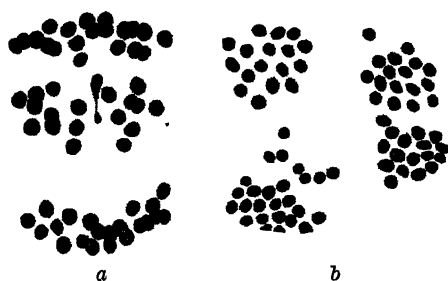


Fig. 7. *F*₁ *Bigelovii* × *suaveolens*, P.M.C., *a*, giant A II, considered to be a product of such an A I distribution as is shown in figures 6*b* and 8*a*, not all chromosomes shown; *b*, T II, distribution fairly regular, although lagging chromosomes occur—not as familiar a condition as that shown in 8*b*.

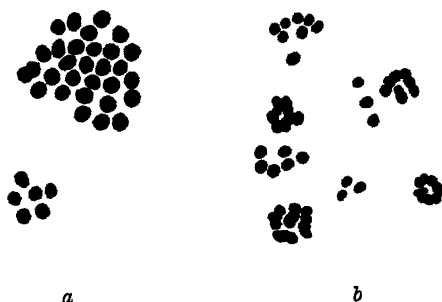


Fig. 8. *F*₁ *Bigelovii* × *glutinosa*, P.M.C., *a*, M II, 30 chromosomes in one plate, 6 in the other; *b*, T II, apparently the result of 2 major homotypic spindles, 1 minor spindle and the presence of a number of lagging chromosomes in the plasma.

It would seem that there would be a considerable possibility of obtaining viable and genetically equivalent gametes in such hybrids as these as the result of equational division at some stage in meiosis and involving the formation of "dyads" in place of "tetrads." With all chromosomes unpaired at A I, a division of the "semi-heterotypic" type might readily occur as a result of a partial failure of the distributional mechanism and the intervention of the time element. No separation of bivalents would need to occur but simply a rounding-up of the univalent mass and a subsequent equational division. We are particularly impressed with the possibility of producing dyads from such an M II condition as is shown in figure 6*b*, and this particular P.M.C. figure was by no means an isolated case. The position of the large plate in the cell and the occurrence of such P.M.C. in company almost exclusively with other homotypic stages and with tetrads would indicate that our interpretation of it as M II is correct. We have also seen one or two similar P.M.C. in which all the chromosomes were in a single M II plate. The regular A II equational division following such a distribution should produce viable gametes containing the haploid sets of both parents, provided that the distributional mechanism at A II was entirely effective. The one case which we have seen in which this condition appears actually to obtain is shown in figure 7*a*. This P.M.C. lay among homotypic stages and tetrads and it seems possible that the giant spindle it contains had its origin in an M II condition such as is shown in figure 6*b*. Apparently both the hybrids under discussion are completely sterile but we have not as yet made any effort to test their fertility by hand pollinations or backcrosses.

The occurrence of complete lack of conjugation in the two haploid sets present in an interspecific hybrid is not, of course, an entirely new or unrecognized situation and has been reported before. It is of interest to us as indicating that other types of conjugation in addition to the Drosophila scheme may be expected to obtain in interspecific hybrids in *Nicotiana* and it may prove to be of some importance as bearing upon taxonomic relationships and upon origins. We propose in the near future to discuss, in this latter connection, the evidence which we have obtained as a result of determinations of chromosome number in a considerable number of *Nicotiana* species and as a result of study of the extent of chromosome conjugation in some 18 interspecific hybrids.

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INTERSPECIFIC HYBRIDIZATION IN NICOTIANA

VI. CYTOLOGICAL FEATURES OF SYLVESTRIS-TABACUM
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INTRODUCTION

On a number of occasions¹ we have described and discussed the data obtained during our long continued studies of *F*₁ *sylvestris-tabacum* and of the backcrosses made upon this hybrid. More recently we have been cognizant of the distinction in chromosome number between the two parent species and have had some knowledge of chromosome behavior in *F*₁ meioses (Goodspeed, 1923).

Some brief recapitulation of our data on these hybrids may be desirable by way of introduction. The *F*₁ uniformly exhibits a striking correspondence in external morphology with the particular *tabacum* variety involved in the cross (Goodspeed and Clausen, 1917), the principal distinction being seen in the enlarged expression of *tabacum* characters in the hybrid. Although completely sterile so far as we know, a little seed can be obtained by backcrossing with either parent. With *sylvestris*, the backcross plants are of three general types: abnormal, almost completely sterile forms; individuals more or less closely approximating the *F*₁; and finally, plants more or less identical with *sylvestris*. This last class exhibits in some cases a considerable degree of fertility, and self-fertilization through a number of generations leads to the establishment of fully fertile races identical with *sylvestris*.

During 1925 we again grew a population of the backcross with *sylvestris*. Of some twenty plants, two were strongly reminiscent of the original *F*₁ and sterile, one was identical with *sylvestris* and fully

¹ Univ. Calif. Publ. Bot., vol. 5, pp. 189-98, 273-92, 301-46; vol. 11, pp. 1-30; Am. Nat., vol. 51, pp. 31-46, 92-101; Proc. Nat. Acad. Sci., vol. 2, pp. 240-44.

fertile, and of the remainder, a few were aberrant forms, completely sterile, and others, less abnormal, showed a varying but uniformly reduced fertility. It is our purpose in the present communication to describe in some detail chromosome behavior and the results of the distributional mechanism operative at meiosis of the F_1 *sylvestris-tabacum* hybrid as indicated, in part, by the chromosome complements of the backcross progeny with *sylvestris*.

CYTOLOGY OF THE F_1

In a preliminary account of the cytology of this plant (Goodspeed, *loc. cit.*), it was stated that the chromosome number in *sylvestris* is 12_{II} ,² in *tabacum* 24_{II} ; that IM^2 in the F_1 both in EMC and PMC showed $12_{II} + 12_I$ and that the bivalents were seen to separate their partners regularly to the poles. The appearance of IT strongly suggested that the univalents divide during or after the passage of the bivalent partners to the poles. No satisfactory evidence, however, as to chromosome number at IIM was obtained. Recently we have published an account (Goodspeed, Clausen, and Chipman, 1926) of the cytology of certain *paniculata-rustica* hybrids in which the haploid parental chromosome numbers are also 12 and 24, and the behavior of the distributional mechanism at IM and IIM of the F_1 corresponded closely to what we knew of the cytology of the F_1 *sylvestris-tabacum* hybrid.

During the past two years we have made a more detailed cytological study of this hybrid from paraffin sections and especially from aceto-carmin preparations. For our present purpose the superiority of the latter is clear; largely, perhaps, because we have had difficulty in finding a suitable fixing agent for stages following interkinesis. In general, our later findings confirm the preliminary account of the cytology of this hybrid, but some modification of certain of the earlier conclusions must be made in the light of the considerable amount of new data in hand.

Figure 1 (*a, b*) exhibits the chromosome complement of *tabacum* and *sylvestris* as seen in polar view at IM. These and all other

² "I" and "II" stand for the first (heterotypic) and the second (homotypic) divisions, respectively. The capital letters which follow these Roman numerals stand, M for metaphase, A for anaphase, and T for telophase. The small subscript Roman numerals "I" and "II" stand for the univalent and the bivalent conditions, respectively.

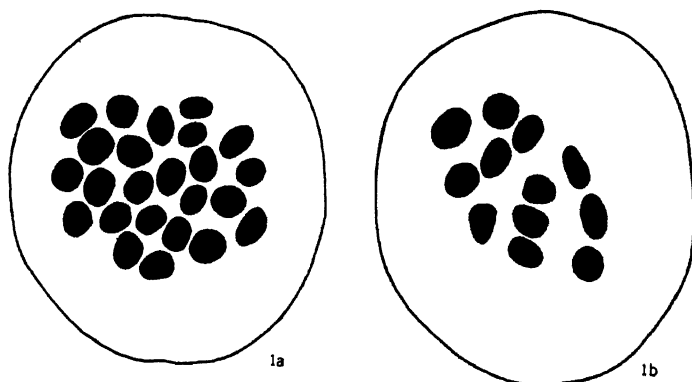


Fig. 1. IM in polar view of (a) *Nicotiana tabacum* var. *purpurea*; (b) *N. sylvestris*, from a crushed aceto-carmin preparation.

figures were drawn from aceto-carmin preparations. Figure 2 shows the characteristic appearance at IM of the hybrid; (a) in side view, (b) in polar view. The establishment of a well organized equatorial plate containing all of the bivalents together with a portion of the univalent complement is of interest to us because it appears that a number of interspecific hybrids in *Nicotiana* do not exhibit any such characteristic equatorial plate stage. We have recently described two such cases (Goodspeed and Clausen, 1927) and know of others. It would appear that the establishment of the bivalent condition in the case of a certain number of chromosomes is essential for the equatorial plate condition at IM shown in figure 2a.

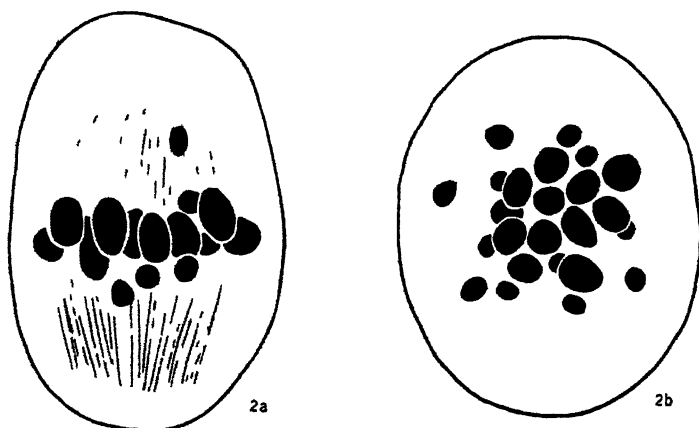


Fig. 2. F_1 *sylvestris-purpurea* IM. (a) Side view from a slightly crushed preparation, only a few chromosomes shown; (b) polar view, complete— $12_{II} + 12_I$.

That 24 chromosomes are present at diakinesis and IM and that 12 of them are larger (bivalents) and 12 smaller (univalents) is perfectly clear. A very large number of these two stages have been carefully studied and we have never found more nor less than 24 chromosomes. At IM varying numbers of univalents may be included in the equatorial plate along with the bivalents and similarly varying numbers may be seen nearer one or other pole. The bivalent condition at diakinesis and IM is as sharply and clearly established in the F_1 *sylvestris-tabacum* as in the F_1 *paniculata-rustica* hybrid.

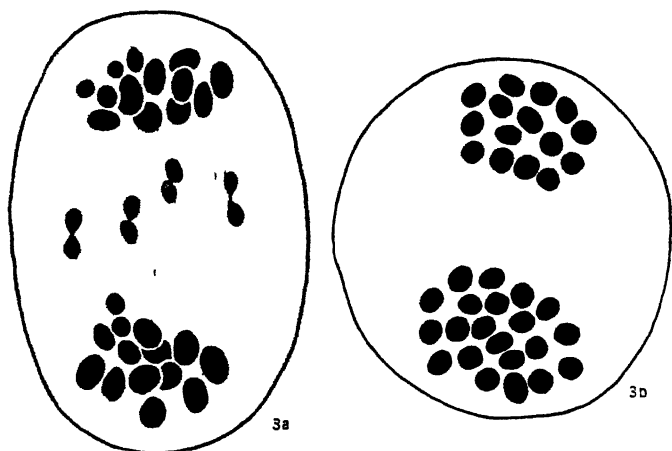


Fig. 3. F_1 *sylvestris-purpurea*. (a) IA; (b) IIM, polar view, fifteen chromosomes in one plate, twenty-one in the other.

In aceto-carmin preparations the apparent division of the univalents at IA is not so sharp as in paraffin material. The condition shown in figure 3a was often encountered but only rarely the situation illustrated in the preliminary account (Goodspeed, *loc. cit.*, p. 473, fig. 1, d^1 , d^2). Comparison of the two types of preparations indicates that the two drawings are quite characteristic for each of them. The distinction may be due to the slower penetration and more gradual fixation in the case of the paraffin material. At any rate, all our experience indicates that aceto-carmin preparations give close approximation of conditions observable in living PMC. In either case there is a suggestion that at least a few of the univalent chromosomes divide at IA, and such behavior was described in the earlier report. At this point it may be stated that we are entirely convinced that usually none of the univalents divide previous to interkinesis; the evidence for this statement will be presented in what follows.

We have made some study in PMC of the fate of the univalents at the end of the first division. It appears from such counts at late IT as we could obtain, and from other data, that lagging chromosomes do not lose their identity but remain intact in the plasma from IA to late tetrad. Chromosomes observed without the daughter nuclei at interkinesis may be interpreted as representing the total number which did not succeed in reaching the poles previous to the rounding up of the daughter nuclei. The following tabulation expresses our data in this connection.

TABLE 1
NUMBER OF CHROMOSOMES IN PLASMA AT INTERKINESIS

Number of chromosomes.....	0	1	2	3	total
Number of PMC	94	39	6	1	140

It appears, then, that in many cases the full chromosome complement after disjunction is included in the daughter nuclei, and our counts at IIM confirm this conclusion.

As to the homotypic series, we have been forced to conclude that none of a large number of fixing agents which we have employed will give entirely reliable results for stages later than interkinesis. Although in aceto-carmin preparations we have seen at IIM some of the irregularities described in the earlier account, they are not numerous. Similarly, the rapidity with which IIA occurs after the close of interkinesis (which is rather long sustained), leads to a large number of early IIA in fixed material and few true IIM. Further, the fixation is usually not precise. We interpret the more or less uniform counts of 19-22, which were reported as IIM and which we have subsequently made, as actual records of IIA where a certain number of chromosomes had divided. In aceto-carmin preparations, on the other hand, the IIM is caught clearly and sharply as distinguished from IIA and we have been able to make a number of counts in none of which more than 36 chromosomes are present.

The counts listed in table 2 are of PMC actually drawn. In addition, a large number of counts from the slide confirm this evidence that an excess of 36 chromosomes does not appear at IIM, and that the undivided univalents are distributed at random in the first meiotic division.

There were a number of PMC at IIM in which we saw one, and a few cases in which we saw two chromosomes, in the plasma. In the

TABLE 2
NUMBER OF CHROMOSOMES AT IIM

Type of distribution	$\frac{12}{24}$	$\frac{13}{23}$	$\frac{14}{22}$	$\frac{15}{21}$	$\frac{16}{20}$	$\frac{17}{19}$	$\frac{18}{18}$
IIM—both plates	$\frac{0}{0}$	$\frac{0}{0}$	$\frac{1}{1}$	$\frac{3}{3}$	$\frac{4}{4}$	$\frac{8}{8}$	$\frac{13}{13}$
IIM—single plates*	$\frac{0}{0}$	$\frac{0}{1}$	$\frac{3}{1}$	$\frac{8}{3}$	$\frac{14}{22}$	$\frac{33}{24}$	$\frac{20}{20}$
Total	0	1	6	17	44	73	66
Calculated	0	1.2	6.7	22.2	50.0	80.0	46.7

* The numerator or denominator in these figures stands for a count corresponding to the numerator or denominator in "type of distribution."

relatively few cases in such PMC where both IIM plates could be counted the total number of chromosomes present was 36, which gives some evidence that where a single chromosome is seen in the plasma at interkinesis it is not half of a univalent. Figures 3*b* and 4*a* illustrate characteristic IIM conditions; the former a rarer case, the latter a more common one.

As already stated, we still recognize that some abnormality is likely to be shown at IIA. We have seen, in aceto-carmine preparations, the two spindles apparently flowing into each other and, rarely, what appear to be giant spindles in which both IIM plates seem to have been united. In the main, however, the IIA situation shown in figure 4*b* is quite characteristic. A few chromosomes, and in a very

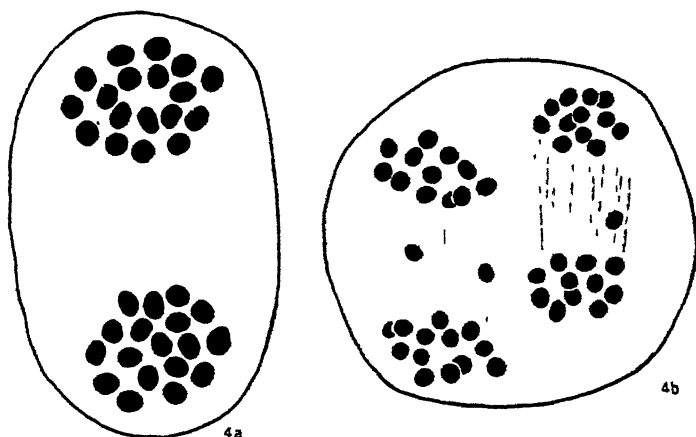


Fig. 4. *F. sylvestris-purpurea*. (a) IIM in polar view, eighteen chromosomes in both plates; (b) IIA showing lagging chromosomes, all chromosomes not included.

few cases what appear to be the entire 12 IM univalents, either lag or seem to have been cast out into the plasma. We have been able in a number of instances to count three or all of the late IIA plates in a single PMC. In three such cases all the plates contained 18 chromosomes, there being no laggards. In some ten other PMC, a total of over 60 chromosomes could be counted, there being four well organized late IIA plates and other chromosomes not included in them.

The end product of the homotypic distributional mechanisms can be judged on the basis of the following summary of counts at IIT.

TABLE 3

NUMBER OF CHROMOSOMES IN THE PLASMA AT IIT

Number of chromosomes	0	1	2	3	4	total
Number of PMC	21	14	10	6	2	53

With reference to chromosome-lagging during IIA, we recognize the importance of the suggestion made in the previous report (Goodspeed, *loc. cit.*, p. 477), that such laggards represent halves of univalents which had divided at IA, but our data as reported above give no support to this proposition. This matter is of decided interest and importance and we propose to study it further in more simplified material.

We have made further study of conditions in EMC but have nothing to add to the previously reported observations, which indicated that PMC and EMC stages are approximately equivalent as to chromosome behavior and distribution.

THE BACKCROSS TO SYLVESTRIS

DESCRIPTION OF THE PROGENY

On a number of occasions we have examined progenies produced as a result of backcrossing F_1 *sylvestris-tabacum* to *sylvestris*, and have described and discussed them in detail (Goodspeed and Clausen, 1922). At that time we were not acquainted with the cytological features exhibited by the parent species or the F_1 . In preceding pages we have described this situation in some detail and in what follows we present a portion of our data on the external morphology, fertility, and chromosome number in a *sylvestris* backcross progeny (25131) grown during the 1925 season.

Of the 21 plants of 25131 brought to maturity, 17 bore white flowers and 4, red or pink flowers. Of the latter class, two plants (P_3 and P_5) were strikingly similar to F_1 *sylvestris-tabacum*. Both were more robust than the true F_1 and differed from each other in flower size, primarily. The following tabulation indicates the situation in this regard.

Description	Corolla	
	Length	Spread
<i>sylvestris</i>	85	43
<i>tabacum</i> var. <i>purpurea</i>	49	36
<i>purpurea</i> ♀ × <i>sylvestris</i> ♂	59	44
25131 P_3	54	44
25131 P_5	57	45

At IM both 25131 P_3 and P_5 showed $12_{II} + 12_I$ (cf. fig. 6a). The distinctions noted above suggest that replacements within the 12_{II} group, producing some *ss*, have occurred as contrasted with the 12_{II} group of the F_1 , which are all *st*. Both plants were sterile.

Plants 16 and 21 bore flowers of a light pink shade and in the latter plant they were abnormal, split, and misshapen. The respective flower sizes were 36–30 and 60–45. Plant 16 was dwarf, much branched, and bore small, fleshy leaves. Plant 21 was tall, stout, with larger leaves, narrow at the base. Both plants were sterile, indeed the majority of the buds were cast so early that it was not possible to obtain significant cytological material from them.

Of the 17 white-flowered plants, a brief description will be given of those the cytological situation in which is figured, and of a few others. Plants 15 and 23 represent, perhaps, the extreme conditions. The former bore the smallest flowers of all the whites, 51–31. It was a semi-dwarf type, stout, with the style short, and generally abnormal in appearance. As shown in figure 6b, at IM there were $12_{II} - 7_I$. Plant 23, on the other hand, appeared to be identical with *sylvestris* in external morphology and at IM showed 12_{II} (cf. fig. 9a). Its flower size was 83–46. Plant 12 was much the same in general appearance but bore shorter flowers—81–45. The stamens were short and the leaf shape decidedly different from *sylvestris*, especially in the narrowness of the leaf base. At IM there were $12_{II} + 1_I$.

Plant 2 bore short-petioled cordate leaves. Its flowers measured 67–40 and at IM there were $12_{II} + 2_I$ (cf. figs. 5b and 8a). In habit, plant 14 was peculiar; not dwarfed, but telescoped in appearance. In flower size it was 75–38 and showed $12_{II} + 1_I$ at IM (cf. fig. 7a). Plant

18 was much branched, with leaves narrow at the base. Its flowers measured 88-39 and at IM there were $12_{II} - 1_I$ (cf. fig. 5a).

From this brief description of a few plants of 25131 it will be seen that this population corresponded in general features to other products of the backcross to *sylvestris* previously grown, and described and figured elsewhere (cf. Goodspeed and Clausen, 1922). There was one plant equivalent to *sylvestris*, another close to this condition, plants comparable to F_1 , and finally a series beginning with plants of a *sylvestris* type and ending in aberrant forms.

CYTOLOGICAL DATA

Our cytological data on 25131 are brought together in the following tabulation. Of 21 plants which came to maturity, the chromosome number at IM was determined in the case of 17.

TABLE 4

NUMBER OF UNIVALENTS AT IM, 25131- 12_{II} IN EVERY CASE

Number of univalents	0	1	2	3	4	5	6	7	8	9	10	11	12
Number of plants	1	5	3	1	1	—	1	1	1	1	—	—	2
Calculated	0	0	0.3	0.9	2.1	3.3	3.8	3.3	2.1	0.9	0.3	0.0	0.0



Fig. 5. F_1 *sylvestris-purpurea* \times *sylvestris* (25131), diakinesis (a) P18, showing $12_{II} + 1_I$, from a crushed preparation; (b) P2, $12_{II} + 2_I$, somewhat later stage than that shown in (a), cell intact.

A portion of our cytological evidence in this culture is illustrated in figures 5-9. The chromosome counts at diakinesis, IM or IIM, were in all cases quite clear, except in the case of P_{10} , where only a small amount of material happened to be available. Diakinesis in aceto-carmine preparations proved to be very acceptable and figure 5 shows

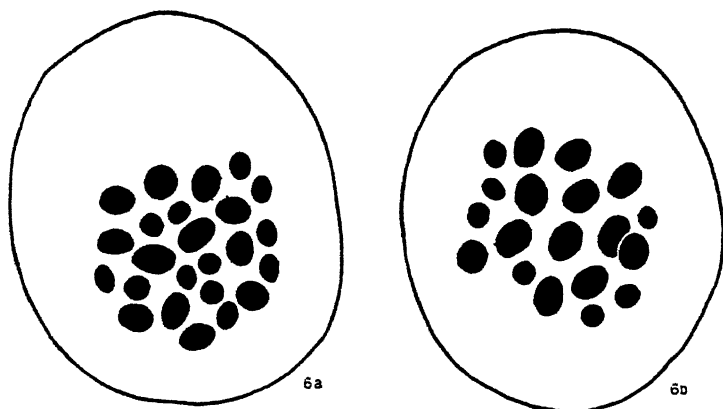


Fig. 6. 25131, IM, (a) P3, showing $12_{II} - 12_I$; (b) P15, $12_{II} - 7_I$.

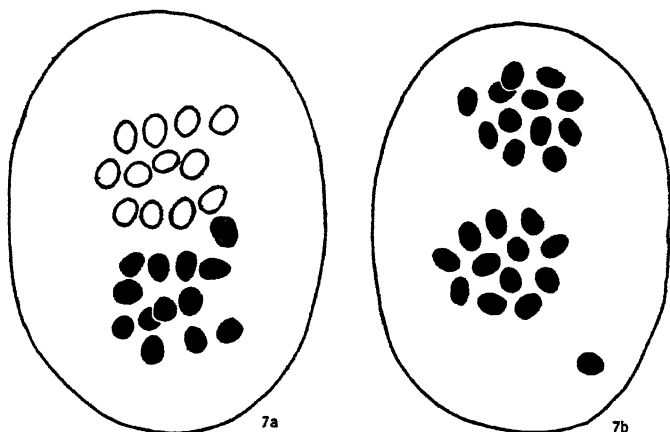


Fig. 7. 25131 P14 (a) IA in polar view showing twelve bivalent chromosomes approaching each pole and the single univalent still near the equatorial zone; (b) IIM in polar view, twelve chromosomes in each plate and the univalent in the plasma as a result of IA lagging.

the situation observed in two of the many plants of which similar stages were drawn. As will be seen, the distinction between bivalents and univalents was sharp. IM counts were also convincing, as indicated by the conditions shown in figs. 6a, b, and 8a. As in diakinesis, the distinction between bivalents and univalents was usually quite clear.

IIM counts were also unusually satisfactory and in all cases were made where any doubt existed as to the number determined from diakinesis or IM. Although we made no study of the point, it is our impression that lagging of univalents at IA, at least as indicated by

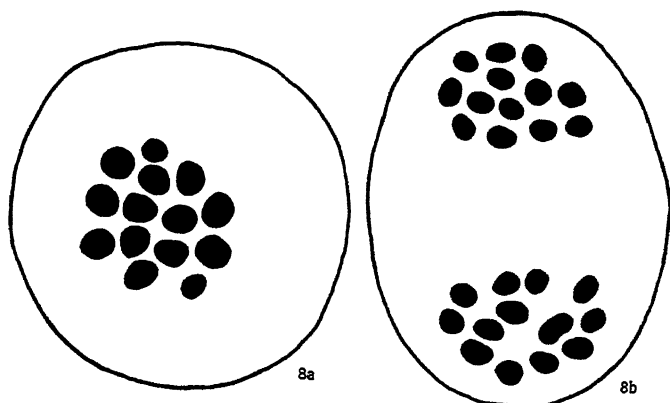


Fig. 8. 25131 P2 (a) IM in polar view, $12_{II} + 2_I$; (b) IIM, polar view, thirteen chromosomes in each plate.

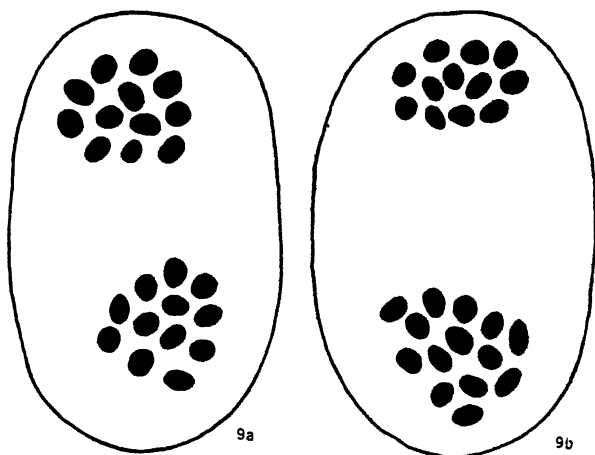


Fig. 9. 25131, IIM polar views (a) P23 showing twelve chromosomes in each plate; (b) P2, twelve chromosomes in one plate and fourteen in the other (cf. fig. 8b).

the presence of chromosomes in the plasma during II, was rare in all cases except plants 3 and 5, for which our statements as to chromosome behavior in the F_1 hold. Figure 7b illustrates the rather exceptional case in which a chromosome, in this case almost certainly the single univalent, was not included in either IIM plate. Figure 7a shows a rather characteristic condition at IA, where the univalent is apparently lagging, but according to our evidence will probably reach one or other pole in time to be included in a daughter nucleus.

In such IIM counts as we have made of $12_{II} + 1_I$ plants—the simplest condition—we have not as yet found indication that the

univalent often divides at IA. Our data on this point are not too extensive, however. Figures 8b and 9a, b, indicate the types of IA distribution with which we are familiar. Some study of the tetrads indicates that, as might be expected from what has been said, a few cells in $12_{II} - 1_I$ plants contain a single microcyte in addition to the four major microspores. We have also on occasion seen two microcytes plus four microspores in such plants.

DISCUSSION

In the previous account the belief was expressed that the twelve bivalents represent $12s - 12t$, the univalents being $12t$. Since Rosenberg's original interpretation of the ten bivalents found at IM in *Drosera obovata*, there have been a number of interspecific hybrids described in which this type of conjugation has been proved to occur. In our own material, for example, we have recently been investigating F_1 *longiflora* ($n - 10$) \times *alata* ($n - 9$) and find at diakinesis and IM $9_{II} - 1_I$ consistently appearing. On the other hand, when the chromosome number in one parental haploid group is twice that in the other and bivalents equal in number to the haploid group of the smaller number occur, only genetic evidence will settle the question as to whether the observed chromosome conjugation is to be interpreted as inter- or intraspecific. Our studies of *paniculata-rustica* hybrids (Goodspeed, Clausen, and Chipman, *loc. cit.*) constitute the first experimental confirmation of the occurrence of the so-called "Drosera scheme" in such cases. The data presented above as to *sylvestris-tabacum* hybrids represent a further case in point.

The cytological evidence in favor of the proposition that in these hybrids the 12_{II} uniformly present in the F_1 at IM represent $12st$, seems particularly clear in view of the lack of chromosome conjugation shown at IM of *tabacum* haploid (cf. Chipman and Goodspeed, 1927). Again, the occurrence of $12_{II} + 11_I$ in F_1 "fluted" *sylvestris-tabacum* hybrids (cf. Clausen and Goodspeed, 1926) bears upon this question. If the 12_{II} of the true F_1 are tt , these F_1 "fluted" hybrids should show $11_{II} + 13_I$. Decisive evidence is furnished by the above-described chromosome situation in 25131, where every plant was found to be of the constitution of $12_{II} - n_I$. If the F_1 condition had been $12tt + 12s$, this backcross progeny, assuming consistent behavior, should contain $12t - xs + ys$, with $x + y = 12$.

The cytological findings for F_1 *paniculata-rustica* backcrossed to *paniculata* indicated that the twelve F_1 univalents were distributed

either at random, giving a gamete series containing from 0-12 univalents in expected frequencies, or in some way by which all the univalents were included in a gamete. Although inconsistent with the high degree of pollen abortion observed in the F_1 , random survival of combinations resulting from the random distribution apparently occurred. All evidence indicates that complete pollen sterility is characteristic of F_1 *sylvestris-tabacum*. This phenomenon is, perhaps, in accord with the evidence furnished by the chromosome garnitures of 25131. The distribution shown in table 4 apparently demonstrates that random distribution of univalents followed by random survival of resulting combinations does not occur. The predominating classes are those in which 0-3 univalents were included, a type which is scarcely represented at all in the *paniculata-rustica* hybrids. The two cases in which all the univalents were included in a gamete provide the other extreme, which was represented by a large class in the other hybrid.

We have found that IM of F_1 *tomentosa* (t') ($n-12$) \times *tabacum* (t) shows $12_{II} + 12_I$. We assume this to represent another case under the "Drosera scheme"—that the 12_{II} are $t't$. Recently we succeeded in backcrossing this hybrid to *tabacum* and the chromosome situation in a progeny of some thirty plants is being determined. Cytologically speaking, the distributional mechanism in F_1 *tomentosa-tabacum* is the same as that in F_1 *paniculata-rustica* and F_1 *sylvestris-tabacum*, and it will be interesting to determine whether the viable gamete combinations correspond to the conditions described elsewhere for the former hybrids or to the situation in the latter detailed above. It is possible that they will consist predominatingly of other combinations.

For a number of years we have been studying genetically and cytologically a series of progenies resulting from repeated backcrosses of *sylvestris* derivatives to *sylvestris*. It is our hope to determine by such studies the effects of the various *tabacum* chromosomes in combination with the full *sylvestris* set and of replacement of *sylvestris* chromosomes by *tabacum* homologs. These studies are being paralleled by corresponding investigations of *paniculata* and *rustica* derivatives of the hybrid involving these two species and it will be possible to initiate a further comparable series of studies of *tomentosa* and *tabacum* derivatives in case certain of the backcross plants from the *tomentosa-tabacum* hybrid, referred to above, behave according to our previous experience in such material and prove to be, in part at least, fertile.

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INHERITANCE IN NICOTIANA TABACUM

VIII. CYTOLOGICAL FEATURES OF PURPUREA HAPLOID

BY

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INHERITANCE IN *NICOTIANA TABACUM*

VIII. CYTOLOGICAL FEATURES OF *PURPUREA* HAPLOID

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INTRODUCTION

Some four years ago two haploid individuals occurred in our cultures of *Nicotiana* and since that time we have been concerned with a detailed cytological study of one of them which we have preserved by vegetative propagation. A first report has been made (Clausen and Mann, 1924) on the origins, external morphology, and, to some extent, the cytology of these two haploid plants. We are here concerned with the *tabacum* var. *purpurea* haploid—23083P57.

This haploid appeared in a population of F_1 *purpurea-sylvestris* and represented a replica on a reduced scale of the female parent of the hybrid. Presumably it arose as a result of proliferation of the egg and so these three, the *purpurea*, the *Datura* (Blakeslee, *et al.*, 1922), and *Triticum* (Gaines and Aase, 1926) haploids represent the only instances of true parthenogenesis in Angiosperms (cf. Sharp, 1926, p. 349). No very detailed description of the cytological features exhibited by any of these haploid plants has appeared and although our data are not as complete as we could wish, it seems desirable to put on record the information at hand, dealing with a type of individual which, from certain cytological points of view, is in a highly simplified condition.

There is little to be added to the original report except on the cytological side. Repeated attempts to secure selfed seed have been unsuccessful, as have attempts to cross with pollen of the diploid parent. These results, together with some cytological evidence that embryo sacs fail to mature, indicate that 083P57 is completely female sterile. On the other hand, some viable pollen is always produced. A few plants have been obtained from *purpurea* diploid ♀ × *purpurea* haploid ♂ and a large population from *purpurea* diploid ♀ × *macrophylla* haploid ♂ (cf. Clausen and Mann, *loc. cit.*). The essential evidence furnished by these populations was that the viable pollen of the haploid contains the full haploid chromosome set of *purpurea*.

METHODS

The data reported on here have been obtained both from paraffin material and from aceto-carminic smears. The latter are extremely valuable, giving the clearest demonstration of stages from second contraction onward; indeed we have examined paraffin material of these mature stages only as a check against the aceto-carminic preparations and for evidence concerning achromatic structures which are not so well brought out in aceto-carminic. We have found the greatest difficulty in obtaining any uniformly favorable preparations after fixation in the case of PMC from IM onward. The effects of some twenty-five killing and fixing agents were compared. The list includes a number of modifications of Flemming's stronger and weaker fluids, some fifteen acetic-alcohol mixtures with formalin and chloroform, and a number of other combinations. In general, we have found that modifications of strong Flemming and Lavdowsky's modification of Merkel's fluid gave best results. Proper fixation of early prophase was more readily secured and in general there was much less difficulty in obtaining proper fixation in all EMC stages. These results apply equally well to all other *Nicotiana* material we have studied.

PROPHASES

In order properly to evaluate the cytological situation in the haploid we made a study of meiotic prophase in *purpurea* diploid (cf. Christow, 1925). In the following descriptions conditions in the two types will be compared.

In the resting PMC of the diploid there is a well defined nuclear membrane surrounding a very delicate, peripherally arranged network of karyotin. The plasmosomes are large, deeply staining, and conspicuously vacuolate. As synizesis approaches, the karyotin assumes a definitely thread-like quality, the delicate leptotene reticulum filling the nucleus in an infinitely tangled condition. We have given special attention to the time at which segmentation takes place and it is our impression that it has not occurred before synizesis, indeed we have seen no evidence of free ends in the pachynema. Similarly, we can find no evidence of doubleness in the leptotene thread system. The withdrawal of the network from the periphery of the nucleus marks the inception of synizesis. Synizesis is formed in the usual fashion, the aggregation of chromatin being of about medium density.

The emerging pachynema (fig. 1) is still highly filamentous but much better defined than the leptonema. At first it is strictly single, uniform, and smooth, with a diameter of about 0.62μ —the nuclear cavity at this period averages 15μ in diameter. A comparison of later stages with this emerging phase leads one to believe that the pachynema keeps on spinning out until it has a diameter of 0.42μ and pairing is already beginning (figs. 1 and 2). These drawings and measurements were made from anthers treated in the same manner, embedded in the same block and stained together. After pairing is introduced it is possible to find many nuclei with both double and single threads. Free ends are present in the system but it is impossible to determine their place of origin. Neither can the number of the segments be accurately determined. Double portions of the pachynema measure 1.25μ in diameter (fig. 3). This indicates that in *tabacum* diploid condensation is subsequent to pairing.

Prophase stages in diploid EMC are often particularly well defined. The post-synizesis pairing proceeds more slowly and regularly and the association appears to be simultaneous among all the pairs of threads. The slenderness of the pachynema is very striking and condensation seems to be somewhat delayed, as compared with PMC. It is apparent that the delicacy of these pachytene stages is approximately as extreme as that of leptonema in many other species and suggests that, from the mechanical point of view, post-synizesis pairing in *tabacum* would fulfil all the requirements of certain genetic postulates.

In PMC, when doubleness has been attained by the entire pachynema (fig. 3), condensation progresses rapidly and second contraction is reached without any conspicuous looping or folding of the shortening and thickening double threads. On the other hand, second contraction is a conspicuous stage and the whole mass of threads becomes much compressed. It is possible that at this stage individual double threads are folded one upon the other.

As shown in figure 3, something approaching a strepsinema occurs at least in a few of the threads but is rarely better defined, and condensation continues until, at mature diakinesis (fig. 4), the chromosomes appear as double droplets, pairs of very short rods or small rings (cf. Christow, *loc. cit.*). These pairs total the haploid number (24) of bivalent chromosomes.

A conspicuous third contraction follows and the now mature chromosomes are so crowded together that it is difficult to distinguish

the outlines of more than a few of them. In EMC it has been difficult to find any evidence of a third contraction, which may simply indicate that it is passed through rapidly in the megaspore mother cell.

In PMC, the seriation of stages in the prophase of the haploid corresponds exactly to the seriation just described for the diploid but the time relations involved are apparently not the same. That is to say, there is a normal synizesis, a second contraction, a diakinesis, etc. (cf. Gaines and Aase, *loc. cit.*, p. 376), but these stages are usually somewhat mixed in a given anther sac instead of showing the usual orderly progression. Further, a distinction between haploid and diploid is always apparent because of the conspicuously smaller quantity of chromatin characteristic of the former. Finally, the behavior of the post-synizesis threads is not the same in the two.

In the haploid the leptotene network is extremely delicate and the plasmosomes, perhaps merely by contrast, seem to be excessively large and well defined. Just as in the diploid, it was not possible to find any evidence of a longitudinal split or a paired condition in the pre-synizesis spireme. In EMC, this stage was very conspicuous, and the continuous nature of the thread and the absence of duality within it were striking. The decrease in the total amount of chromatin characteristic of the haploid was of no great assistance in a study of this stage because of the characteristically interwoven condition of the thread. At synizesis, however, the smaller total volume of chromatin was exhibited in the relatively small size of the closely contracted spireme—the nuclear cavity is 8.25μ in diameter.

From synizesis onward, anther sacs often show an entire absence of the orderly seriation exhibited by the diploid. One may find pachynema persisting at the tips and even about the entire periphery while throughout the remainder of the sac there is a confusion of second contraction, diakinesis, and even IM and T. After a detailed study of these conditions we have constructed the following series which appears to fit the situation.

As in the diploid, the pachynema (fig. 5) is not a collection of individual threads but is still a continuous delicate but well defined thread. This is perfectly clear when sections are cut thick enough to include an entire nucleus. A tangled thread system appears which cannot be followed throughout its entire length because of fusions at points of intersection. On the other hand, in very thin sections, loops and even loops twisted upon themselves, appear, strongly suggesting pairing of chromosome lengths. The pictures of entire nuclei, together

with the fact that no bivalents appear at diakinesis, correct this impression and serve to emphasize the danger of interpretations based entirely or largely upon thin sections of early and mid-prophase stages.

As condensation progresses, individual chromosome lengths become better distinguished (fig. 7). Ultimately it is completed and the chromosome lengths pull apart, leaving delicate connecting filaments trailing behind (fig. 8). In some cases, and especially in EMC, it is possible to determine that these distinct or almost distinct chromosome lengths total twenty-four.

A characteristic second contraction (fig. 9) follows at once and consists of a simple massing of chromosome lengths with no evidence of pairing. As the massed chromosome lengths move apart, they contract to short, thick rods which lie about the periphery of the nucleus (fig. 10). They are not associated in pairs at this diakinesis, there are twenty-four of them, and they show no sign of a lengthwise split (fig. 11).

LATER STAGES

In diploid *tabacum*, IM (fig. 12) consists of twenty-four bivalents appearing as single large droplets with few size distinctions within the group. The equatorial plate is very sharp except that one bivalent has a tendency to disjoin prematurely (fig. 13). With this exception, the bivalent partners ordinarily pass to the poles in orderly rows (fig. 14), the individual members giving almost no evidence of a split until early telophase. Only very rarely does one see lagging of one chromosome at IA. At interkinesis the longitudinal split is very conspicuous and because of the large size of the daughter nuclei, this stage in partial sections might conceivably be mistaken for diakinesis. The split is entirely closed before IIM, where a total of forty-eight chromosomes can be counted in the two plates. As to tetrad formation, there is nothing to be added to Farr's (1916) description of the process.

Chromosome behavior in the haploid at corresponding stages is strikingly different. In the first account of the occurrence of *purpurea* haploid, some description of chromosome behavior at the meiotic divisions was included (Clausen and Mann, *loc. cit.*, p. 123). Further investigation has failed to confirm many of the observations there noted. Measurements give no evidence that each of the twenty-four chromosomes which appear at what corresponds to early IM is the

size of one member of a pair in diploid *tabacum*; ordinarily, at no stage earlier than IT do they split longitudinally, nor do they resemble somatic chromosomes of the diploid. These and other points will be referred to in what follows.

The sequence of events included in the period from third contraction to what corresponds to IA explains the peculiar position of the chromosomes on what appears to be the mature spindle. Just as in the case of F_1 *Bigelovii-glutinosa* (Goodspeed and Clausen, 1927), we are here impressed with the disturbance exhibited in late meiotic periodicity.

The mature bipolar spindle often forms, and makes possible a random distribution of the twenty-four univalents, corresponding in time consumed to the distribution of bivalent partners in the diploid IA. Even when complete bipolarity is attained previous to the start of distribution, the chromosomes commonly lie on the spindle in the positions which they assumed under the influence of the multipolar spindle following the opening out of third contraction (figs. 15 and 16). There are also cases (fig. 17) in which a quite distinct equatorial plate of univalents is formed, reminiscent, perhaps, of the equatorial plate of univalents which has been seen to form in the canina roses (Täckholm, 1922), etc., following the passage of bivalent partners toward the poles.

It is possible that such a true metaphase with all the twenty-four chromosomes in an equatorial plate may always precede the situation described above in which the chromosomes lie all along the axis of the bipolar spindle. If this is the case it is an exceedingly transient stage. Widely varying conditions from anther to anther and from one PMC to another suggest that no such uniformity at any stage occurs. In paraffin material an equatorial plate which includes over half the univalents occurs often. In aceto-carmine preparations it is less frequently seen (fig. 18). It is clear, however, that at what corresponds to IM in the diploid, the spindle may in many PMC be of such a character as to permit random distribution of the twenty-four univalents which it holds.

On the other hand, we have seen many PMC in which meiotic periodicity has been disturbed to such an extent that bipolarity is not attained until after the period at which disjunction or distribution would normally have taken place. In such cases, as in F_1 *Bigelovii-glutinosa*, chromosomes are held in peaks of the multipolar spindle which has only in part become bipolar (fig. 15). Along the axis of

this bipolar portion distribution apparently is going on while the remainder of the chromosomes are held near one or the other pole to be included ultimately within one or the other daughter nucleus. The result of such chromosome distribution is shown at IIM, where counts indicate that in a number of cases a majority of the univalents were included in one daughter nucleus (fig. 19).

In the majority of cases 24 chromosomes can be counted during this period which represents IM to A. There are, however, some PMC in which a few "bivalents" appear (fig. 16). A group of 21 counts of PMC in a strictly IM condition showed 18 with the full 24 chromosomes, 2 with 23, and 1 with 20. Where some "pairing" has taken place it appears that the size of the chromosome pair is approximately equivalent to that of a bivalent in the diploid. On the other hand, most of the univalents at IM to A are larger than one member of a bivalent in the diploid. This is particularly true when the size of the univalents in the haploid is compared with that of the univalents passing to the poles at IA of the diploid (cf. figs. 14 and 15). It seems clear that the occurrence of "bivalents" at IM is not a reflection of pairing before third contraction because no evidence can be found of bivalents at diakinesis or earlier. We suggest that the very close association of the chromosomes during third contraction had produced in some cases varying amounts of adhesion of the twenty-four univalents. Such a purely mechanical effect should produce other multiples in addition to bivalents and in a number of cases groups of three chromosomes have been seen. We have no data as to the fate of such "bivalent" or "trivalent" chromosomes as occasionally occur—whether they "disjoin" normally or pass to the poles as bivalents, separated or still united.

As noted in the previous report, division of the univalents sometimes occurs. A portion of our data on this point is given in the following tabulation.

Number of chromosomes dividing at I	0	1	2	3	4	5	6	7	8			24	Total
Number of PMC	48	7	4	2	3	1	4	5	2			4	80

The univalents apparently undergoing division are not necessarily those which appear to be lagging at early IA, as stated in the previous report, but may be found in a variety of positions on the spindle (fig. 20). We have demonstrated in other material that evidence of chromosome division at I is not necessarily significant, the homotypic

counts indicating that these divisions are only occasionally completed. That this is not necessarily true for 083P57 is shown by the IIM counts listed below.

Since the fertile pollen of *tabacum* haploids has been shown to contain the full haploid set of *tabacum* chromosomes, and since considerable numbers of dyads in place of tetrads occur in more mature anthers, the extent and manner of occurrence of chromosome division become of special interest and importance. In the first place we have only rarely (cf. tabulation above) seen all twenty-four chromosomes dividing simultaneously, the halves apparently preparing normally to pass to the daughter nuclei (figs. 24 and 25). As in the case of the haploid wheat (Gaines and Aase, *loc. cit.*, p. 377), it is possible that separation of the respective halves to opposite poles does not always occur. In our material the formation of an equatorial plate including all or practically all the univalents appears uniformly to precede simultaneous division of all the chromosomes whereas, in the haploid wheat, division occurs while the chromosomes are lying along the spindle. That successful division of all twenty-four chromosomes does occur is indicated by counts of twenty-four in single interkinesis plates (fig. 23) and at IIM (fig. 21) as shown below.

It is clear that such division of all the univalents in IA largely accounts for the formation of dyads and the production of viable pollen grains containing the full haploid set of *tabacum* chromosomes. In the corresponding situation in *Datura* haploids it is assumed that a homotypic or "non-reductional" division simply replaces the heterotypic or "reductional" one. In 083P57 we have evidence that the "heterotypic" division is not replaced but is suspended in a manner recalling the "semi-heterotypic" division of Rosenberg (1917). Apparently the first spindle rounds up (fig. 22), possibly to the extent of the formation of a nuclear membrane, and the chromosomes when liberated form an equatorial plate (figs. 24 and 25). Possibly something of this same sort occurs in the haploid wheat where the entire chromosome group "may cohere in an irregular mass in the center of the cell" (*loc. cit.*, p. 377). It is possible that late establishment of bipolarity in the spindle (cf. p. 146) may be responsible for such suspensions of the first division. We have some evidence that the intervention of the sustained multipolar spindle might even result in the accumulation of all the twenty-four chromosomes at one pole (cf. Goodspeed and Clausen, *loc. cit.*).

Few PMC showed chromosomes in the plasma at interkinesis, as indicated in the following tabulation.

NUMBER OF CHROMOSOMES IN PLASMA AT INTERKINESIS

Number of PMC

0	1	2	3	4
122	13	5	2	1

In one or two cases the laggards appeared to be initiating or undergoing division (fig. 26a). At interkinesis all chromosomes were conspicuously split just as in the diploid at this stage (fig. 23).

In aceto-carmin material homotypic stages are very clearly shown. This has uniformly been the case in *Nicotiana* species and hybrids which we have studied as has the lack of definition in such stages in paraffin material. Our data for PMC as to chromosome number at IIM are given in the following tabulation.

Type of distribution	$\frac{4}{20}$	$\frac{5}{19}$	$\frac{6}{18}$	$\frac{7}{17}$	$\frac{8}{16}$	$\frac{9}{15}$	$\frac{10}{14}$	$\frac{11}{13}$	$\frac{12}{12}$	$\frac{9}{16}$	$\frac{12}{14}$	$\frac{13}{14}$	$\frac{14}{14}$	$\frac{14}{15}$	$\frac{13}{16}$	$\frac{24}{24}$	Total
Number of PMC	1			1	3		2	4	3	1	1	1	1	1	1	1	21

As indicated above, there is here evidence that in a number of cases the division seen in IA chromosomes is actually completed and that halves of univalents pass to the poles along with the undivided univalents (fig. 20).

A large proportion of the early IIA examined appeared normal in general details. At IIT, however, chromosome lagging was seen in many cases (fig. 27). Apparently most of the laggards are ultimately included in one of the granddaughter nuclei because there is no corresponding proportion of microcytes or micronuclei in the tetrads. The number of PMC in which late IIA lagging was observed was much greater than that in which chromosome division at IA was seen, which suggests that lagging at II is not confined to chromosomes which had previously undergone division.

We have seen a number of cases in which the two IIM were apparently fused to form a combined spindle. From such a fusion a dyad with the full haploid set of chromosomes could conceivably be produced. Close examination of such cases seems, however, to indicate that the fusion is not complete and that the two metaphases are merely lying at approximately the same level in the cell and oriented in the same plane without a true fusion of spindles (fig. 28). Cytokinesis in such a case would presumably result in tetrad rather than dyad formation.

The greater proportion of tetrads contained only four cells of approximately equivalent size, the nuclei of which lost a considerable proportion of their staining capacity before separation of the microspores was completed. Our data as to the tetrad stage, not including the occurrence of microcytes or micronuclei, are summarized in the following tabulation.

Number of cells	2	3	4	5	6	Total
Number of PMC	18	34	692	31	12	787

It seems fair to assume that the majority of the dyads counted represent cases in which all the twenty-four univalents divided at IA (figs. 24 and 25).

Stages from diakinesis to interkinesis have been studied in EMC. The former is very conspicuous and counts gave no evidence of any pairing. All the early first divisions were of the type shown in figures 15 and 18. A curving of the spindle is common and the chromosomes lie well separated along its course. Distribution did not appear to be as uniformly successful as in PMC, a number of chromosomes often lying in the plasma at IT. Neither in these laggards nor in chromosomes at earlier stages of I was division observed. This point was checked by a number of counts at IT which gave a total of only twenty-four chromosomes at the two poles.

EMC homotypic stages were not clearly shown in paraffin preparations. In a number of cases degeneration in both cells was obviously under way, while in others it was still possible to see the two spindles, which usually were not normally oriented. So far as we know, development in EMC rarely goes beyond the two-cell stage.

We have seen no indication of the abnormalities in sporogenesis reported for the haploid wheat. In using the aceto-carmin method large numbers of anthers were examined but no suggestion of fusion between PMC was observed.

DISCUSSION

We have been particularly interested in chromosome behavior in *purpurea* haploid because of its relation to genetic and cytological data which we have been accumulating on species hybrids involving *Nicotiana tabacum*. Largely on the basis of genetic and to a lesser degree on the basis of cytological data, we have contended (Goodspeed, 1923; Clausen and Goodspeed, 1926) that the $12_{II} + 12_I$ which

appear at IM of F_1 *sylvestris-tabacum* represent 12st - 12t. This contention is sustained by the absence of pairing within the haploid *tabacum* set described above. Apparently F_1 *sylvestris-tabacum* is the first instance in which the occurrence of the "Drosera scheme," in hybrids one parent of which has twice the chromosome number of the other parent, has been proved by a combination of genetic and cytological data on the F_1 plus evidence of lack of pairing in the haploid set of the parent possessing the larger chromosome number. The cytological evidence of lack of pairing in the haploid *tabacum* set is also important in its application to any conjugation phenomena which occur in species hybrids involving *tabacum* as a parent.

Where it is possible to deal with a haploid rather than a diploid chromosome set in meiotic prophases, evidence on certain matters of some fundamental cytological significance should be more readily obtained. Since the chromosome initials of the haploid remain single from the inception of synizesis to the first metaphase and those of the diploid become double between synizesis and second contraction, the time of pairing of paternal and maternal elements in *tabacum* is established. As indicated above, we are convinced that in *Nicotiana tabacum* at least, post-synizesis rather than pre-synizesis pairing is the rule. In particular, we can deny the existence of a lengthwise split in the pre- or early post-synizesis spireme, the simplified condition of the material making possible a categorical answer to this question. Doubleness of meiotic prophase threads in this species can thus be assigned to significant pairing of univalents. As we have indicated, various features described as characteristic of certain telosynaptic stages are to some extent seen in the haploid prophases. Their occurrence in such material, where the earlier and later stages indicate lack of pairing, suggests that their significance is not so great as has been claimed.

We are making a separate study of somatic mitoses in 083P57, particularly of the occurrence of cells containing diploid and higher chromosome multiples. Our data on these points will be published in the near future.

In diploid *tabacum* it was not possible to find evidence of chromosome tetrads at early or mature diakinesis. It seemed possible that the more simplified condition at these stages in the haploid would give evidence of the longitudinal split. We were unable, however, to see the occurrence of the split any earlier than in the diploid, where it is first evident at IA.

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EXPLANATION OF PLATES

Figures in the accompanying plates were drawn with the aid of a camera lucida, using a Leitz mono-objective binocular microscope, no. ABM, with Zeiss, 2 mm., N.A. 1.4 objective and 15x eyepiece.

PLATE 4

Nicotiana tabacum var. *purpurea*—diploid, PMC. Paraffin material

Fig. 1. Single spireme following emergence from synizesis.

Fig. 2. Paired and unpaired threads. Very thin section.

Fig. 3. Doubleness has been attained throughout the pachynema. Very thin section.

Fig. 4a, b. Diakinesis; twenty-four bivalent chromosomes.

N. tabacum var. *purpurea*—haploid, PMC. Paraffin material

Fig. 5. Single spireme following emergence from synizesis.

Fig. 6. Pachynema. Segmentation is beginning. Free ends may be due to cutting.

Fig. 7. Segmentation. Twenty segments can be distinguished.

Fig. 8a, b. Delayed segmentation. Chromosome lengths total twenty-four.

Fig. 9a, b. Second contraction, a simple massing of chromosome lengths with no evidence of pairing.

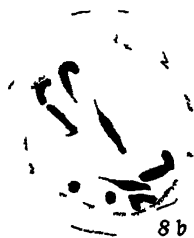


PLATE 5

N. tabacum var. *purpurea*—haploid, PMC. Paraffin material

Fig. 10*a, b*. Early diakinesis. That the apparent association in pairs is not a reflection of the real condition obtaining becomes apparent from the following figure.

Fig. 11*a, b*. Diakinesis with twenty-four univalents.

N. tabacum var. *purpurea*—diploid, PMC. Paraffin material

Fig. 12. IM, with twenty-four bivalent chromosomes.

Fig. 13. Very early IA. Note early separation of chromosomes at the right.

Fig. 14. Late IA.

N. tabacum var. *purpurea*—haploid, PMC

Fig. 15. Multipolar spindle, with twenty-four univalent chromosomes. Aceto-carmin material.

Fig. 16*a, b*. IM; the position of the chromosomes reflects the multipolar spindle. This figure is offered as one of the rare examples of mechanical pairing (cf. p. 147). Paraffin material.

Fig. 17. Polar view IM. All save three of the chromosomes lie on the plate. Aceto-carmin material.

Fig. 18. Side view IM or IA. A familiar type, following multipolar spindle, possibly with omission of an equatorial plate. Aceto-carmin material.

Fig. 19. Polar view of IIM. One plate with eight, the other with sixteen chromosomes. Aceto-carmin material.



a

10



b



a

11



b



12



13



14



15



a

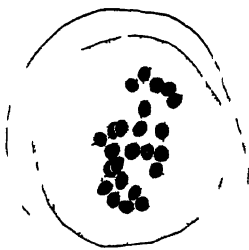
16



b



17



18



19

PLATE 6

N. tabacum var. *purpurea*—haploid, PMC

Fig. 20. Late IA. Four univalents have completed division as indicated by the number of chromosomes present and four are in the process of dividing. Aceto-carmin material.

Fig. 21. IIM, with twenty-four chromosomes on each plate. A result of the situation illustrated in figures 24 and 25. Aceto-carmin material.

Fig. 22. IA rounding up to form one nucleus with twenty-four chromosomes. Aceto-carmin material.

Fig. 23. Interkinesis, with twenty-four split chromosomes in one daughter nucleus. Aceto-carmin material.

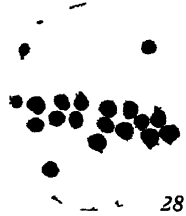
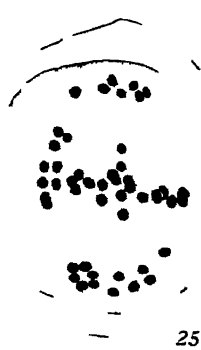
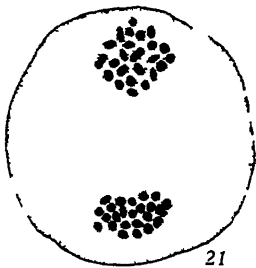
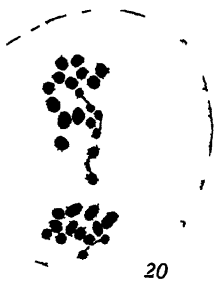
Fig. 24. A giant spindle showing division of the univalents on an equatorial plate. One chromosome not yet dividing. Aceto-carmin material.

Fig. 25. A giant spindle; distribution of halves of univalents under way. Aceto-carmin material.

Fig. 26a, b. Early interkinesis. A lagging chromosome, in a partially divided condition, in the plasma. Paraffin material.

Fig. 27. IIA. Lagging chromosomes in the plasma. Aceto-carmin material.

Fig. 28. Parallel arrangement of homotypic spindles giving rise to a condition readily confused with a giant spindle (cf. figs. 24 and 25).



CHROMOSOME NUMBER AND
MORPHOLOGY IN NICOTIANA

I. THE SOMATIC CHROMOSOMES AND
NON-DISJUNCTION IN *N. ALATA*
VAR. *GRANDIFLORA*

BY

MABEL L. RUTTLE

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INTRODUCTION

The genetics and especially the cytology of interspecific hybrids in *Nicotiana* have been subjects of investigation here for a number of years.¹ As these studies have progressed the need has developed for further knowledge of the number and morphology of chromosomes in the parental species involved. It is proposed to publish a series of articles on this subject, the present note being the first of the series. Acknowledgment is made to Professor T. H. Goodspeed for assistance and criticism during the work.

Nicotiana alata var. *grandiflora* has been grown in the University of California Botanical Garden for many years under a variety of numbers referring to the various sources from which apparently equivalent seed has from time to time been received. Extensive cultures are now under investigation from a genetic as well as from a cytological point of view, so that ultimately a full discussion of its taxonomic status will be possible. Here, reference is made only to Setchell's (1912) description of the species. The plants, from which the material used in this study was obtained, were grown in the greenhouse under the number U.C.B.G. 065. The original seed of this number was collected in 1923 from a garden in Berkeley.

The haploid chromosome number of *alata* was recorded by Goodspeed (1923) as nine. At the same time, however, he found evidence that in PMC non-disjunction was occurring, as indicated by counts of eight and ten in IIA. His figures of this stage also showed two chromosomes apparently larger than the others. Christow (1925), on the other hand, reports that the haploid chromosome number of *alata*

¹ Univ. Calif. Publ. Bot., vols. 5 and 11, and elsewhere.

is eight, and that the somatic number as counted in ovary walls is sixteen. His figures however do not offer convincing evidence as to the correctness of his counts, a point which will be referred to in what follows.

MATERIAL AND METHODS

Material of EMC, PMC, and root tips used in this study was fixed in Taylor's modification of Flemming's solution (Taylor, 1925), and in a modification of Karpechenko's chromacetic formalin solution. The latter was made up in two solutions which were mixed in equal proportions as used. The first contained, water 90 cc., acetic acid 10 cc., and chromic acid 1 gr., and the second, formalin 40 cc. and water 10 cc. This was convenient to use and gave uniformly good fixation of root tips and ovaries and fair fixation of PMC. Material was embedded in paraffin in the usual manner. Sections were cut 6–10 μ in thickness, principally 8 μ , and were stained in Haidenhain's iron alum haematoxylin.

Chromosome counts were made from paraffin sections of EMC and PMC and in greater numbers from PMC smears stained with Belling's iron aceto-carmin, with and without iron. Some counts were also made from smears fixed and stained by Taylor's (1924) smear method and by Kornhauser's (1926) method. While these had the advantage of being permanent mounts, they were not generally so satisfactory as the Belling mounts. An endeavor was made to run the Belling mounts up in alcohol, clear, and mount in Canada balsam, according to Belling (1926), but while sometimes good results were obtained, this was not uniformly the case.

CHROMOSOME NUMBER AND MORPHOLOGY IN SOMATIC CELLS

The somatic chromosomes of *Alata* were studied in the metaphase and anaphase stages of mitosis obtained from rapidly growing root tips and ovary walls of several plants. In the root tips, cross-sections gave the most satisfactory division figures for study, although longitudinal sections were useful for comparative purposes. The figures were chosen from the differentiating primary cortical tissue lying in the transitional zone between the regions of rapid division and elongation on the one hand, and between the differentiating exodermis and endo-

dermis on the other. Here the primary cortical tissue is only four or five cells in thickness, and having begun vacuolation contains large cells with a comparatively loose cytoplasmic structure. Since the cells are large, the chromosomes are well separated on the equatorial plate and the cytoplasm, being less dense than that in the smaller celled and more actively dividing meristematic tissue, the chromosomes have sharper outlines.

In the ovary walls the cells, although having a fairly loose cytoplasmic structure, are much smaller and less regular in shape than those of the root tip. The chromosomes at metaphase are consequently

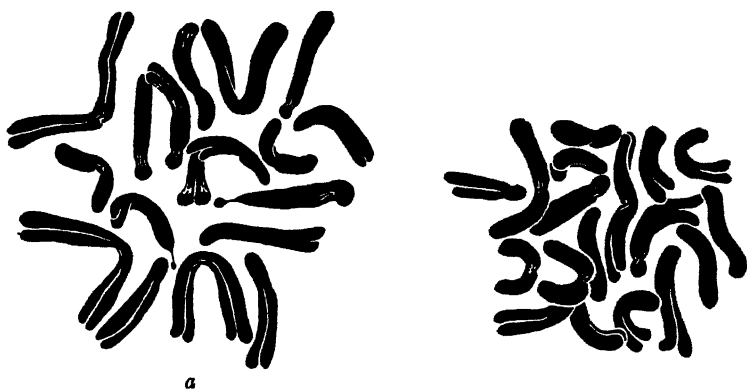


Fig. 1. *Nicotiana glauca* var. *grandiflora*, somatic mitoses, polar view, equatorial plates. *a*, root tip, showing four long, two satellited, and twelve medium sized chromosomes, two of which are probably satellited. *b*, ovary wall, showing four long but no satellited chromosomes. $\times 4500$.

grouped much more closely, so that although fixation seemed good and the stain sharp and clear, the plates were never so flat nor so open as in the root tip preparations. Moreover, as has often been recorded (Hance, 1918, and others), the chromosomes appeared shorter and thicker than in the root tips and exhibited a less definite chromosome morphology.

Approximately fifty equatorial plates in somatic cells were carefully counted and in more than twenty-five of them an entirely satisfactory count on the basis of careful camera lucida drawings could be made. From this evidence it is clear that the somatic number in *glauca* is 18 (figs. 1*a*, *b*; 3*a*). The chromosomes appear as small, slender, sometimes straight, but more often curved rods with rounded ends. Many chromosomes at metaphase show the longitudinal split, which may be seen only at the end, or may be followed the entire length of the chromosome.

It has been possible to arrange the chromosomes into three main groups on the basis of chromosome length and the presence of satellites. In group 1 there are four chromosomes which have a marked tendency to appear Γ -shaped. Each one of them is consistently at least one-third longer than any of the other somatic chromosomes. This size distinction is clearly brought out in figures 1 and 4. In all four chromosomes a median or nearly median constriction is often evident. The position of the chromosomes on the spindle, as viewed in longitudinal section (fig. 2a) and their mode of separation at anaphase, indicate that this constriction is probably the point of spindle fiber attachment. Also, one arm of these chromosomes often

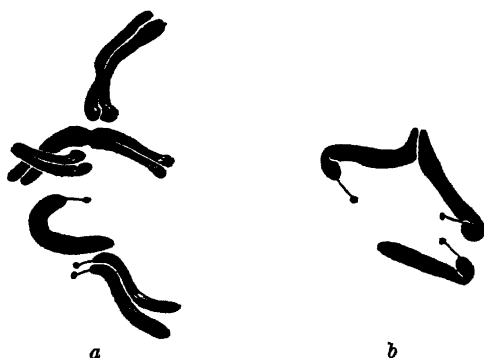


Fig. 2. *N. alata* var. *grandiflora*, somatic mitoses from root tip, side views. a, characteristic position of various chromosomes on equatorial plate. b, satellited chromosomes at anaphase. $\times 4500$.

has a marked submedian or subterminal bend, at which point a second constriction is frequently seen. This flexure was apparent in only one of the four long chromosomes in figure 1a, but has been observed in at least three of these long chromosomes in other division figures.

Unstained areas, resembling transverse scissions, are sometimes seen in some of the chromosomes on the equatorial plate (fig. 3c). The number of these scissions and their size is increased by applying slight pressure to the coverslip. If further pressure is applied, the chromosomes are easily broken apart at these points and, indeed, were it not for the delicate peripheral lines joining the chromosome portions, the clear areas might be regarded as breaks. In the long chromosomes two such unstained areas (fig. 3c B) may be seen which correspond in their locations to those of the constrictions described above.

Such unstained areas in the chromosomes have previously been observed by several workers. Martens (1922) figured and described

in some detail in *Paris quadrifolia* "*scissions transversales*" which at first sight appeared to divide the chromosomes into "*deux tronçons séparés*." The portions, however, as in *alata* were actually united by "*un élément périphérique continu*." Martens considered the true nature, origin, and function of these "*scissions transversales*" uncertain, although he thought they were probably of some importance in an interpretation of chromosome structure, and quite certainly not artifact. In *alata* their origin and significance is likewise uncertain. It may be that these areas are an intrinsic part of the chromosome

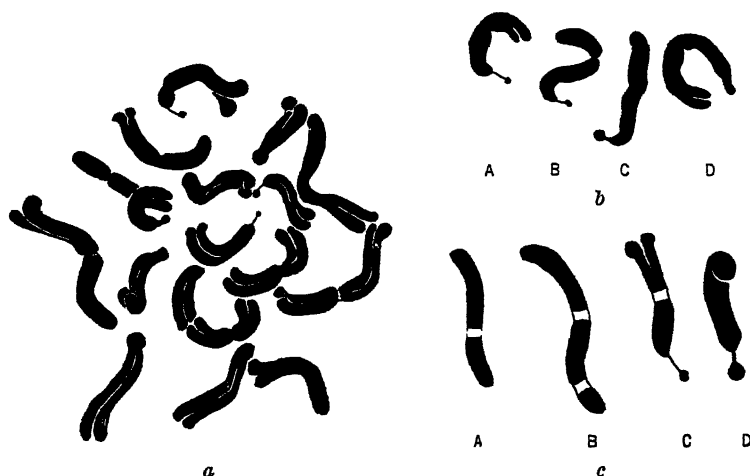


Fig. 3. *N. alata* var. *grandiflora*, somatic mitoses from root tip. *a*, equatorial plate showing three chromosomes each bearing a small proximal satellite, and a fourth chromosome with a knob-like end which may represent a large satellite. *b*, four chromosomes, *A*, *B*, *C*, *D*, from an equatorial plate, *A* and *B* bearing small satellites, *C* bearing a large satellite, and *D* indefinite. *c*, three chromosomes, *A*, *B*, *C*, from two equatorial plates showing the transverse clear areas and a fourth, *D*, showing a large satellite. $\times 4500$.

structure, which after fixation are usually contracted so as to appear as simple constrictions. This seems more probable in view of the fact that their location, particularly in the long chromosomes, corresponds with the previously observed position of the constrictions (figs. 1*a*; 2*a*; 3*a*; 3*c* *B*).

Group 2 requires further study. It is made up of at least one pair of satellited chromosomes and probably of two pairs. If fixation is poor, if the sections are slightly over or understained, particularly the latter, or if the chromosomes, as often happens, are grouped too closely at the equatorial plate, the satellites do not show. Nor were they found on chromosomes in division figures in the ovary walls, a metaphase from which is shown in figure 1*b*. This, however, does not

necessarily constitute a fundamental difference in the two types of tissue, but may be due to the difficulty already referred to, of obtaining well separated plates.

The size, number, and attachment of the satellites is still very uncertain. Two chromosomes bearing small proximal satellites well separated from the main body of the chromosome have been repeatedly found. These are shown in figures 1*a*; 2*a*, *b*; 3*a*, *b*. In figure 1*a*, to be sure, there is an apparent difference in satellite size, but this figure was chosen to represent the whole group of chromosomes lying flat, and the satellite on the bent chromosome was curved under in such a way that its full size may not have been evident. A third satellite has been found often enough to be fairly certain that there are at least three satellited chromosomes. Up to the present, four certain satellites have not been found in one cell although, as figures 3*a* and 3*b* show, indications of what may be a fourth have been found a few times. The appearance of the suspected fourth satellite (fig. 3*a*, *b* *D*, *c* *D*) suggests that it may be a large satellite and that it may often be either contracted to the chromosome or oriented so as to appear as a knob at the end of the chromosome. The same also may be true of the suspected third satellite (fig. 3*b* *C*) although often, as in figures 3*a* and 4*a*, the three apparent satellites are small. On the other hand, one unquestionably large satellite (fig. 3*c* *D*) was found in the side view of a metaphase, and indicates the presence of at least one large satellite. This same uncertain condition as regards number and size of satellites has been found in root tips obtained from several different plants, all apparently normal. It is hoped that further study will reveal the true satellited condition.

The position of the spindle fiber attachment on the satellited chromosomes has not been ascertained with any degree of certainty, although the evidence indicates that, on at least one pair of chromosomes, it approximates median, being perhaps a little nearer the end bearing the satellite. The chromosomes were seldom found lying as flat as the one chromosome in figure 1*a* but were usually bent in the form of a *J* or a *V* (figs. 2*a*, *b*; 3*a*). The orientation and possible spindle fiber attachment of various chromosomes, chosen from two metaphase plates, are shown in figure 2*a*. Here, as was almost always the case, the satellited chromosomes are bent in such a way that the arm bearing the satellite is directed inward. In only one case was a satellite found on the free arm of the chromosome, but the chromosome halves in beginning separation may have rotated to this position.

In figure 2*b*, a side view of an anaphase, two satellited chromosomes are seen approaching the poles. The arms bearing the satellites are sharply bent and are directed toward the poles. On account both of the bend and the grouping of the chromosomes at the poles, it is difficult to locate the satellites at this stage. From this figure it would appear that the fiber attachment is nearer the satellited end. On the other hand, the constrictions in chromosomes *A* and *B*, figure 3*b*, and the clear area in chromosome *C* figure 3*c* are evidently median. It may be that, if there are two pair of satellited chromosomes, the attachment is not the same for both pairs.

Group 3 consists of the remaining five or six pairs of medium sized chromosomes. This group is fairly uniform in size and appearance, but it seemed worth while to make a considerable series of measurements in the hope that on the basis of length a means of individual identification would be found. It was also hoped that the measurements would prove useful as a basis of comparison with other species to be studied later.

For making measurements a modification of the methods of Hance (1917, 1918) was followed. Sixteen of the flattest metaphase plates from the primary cortical region of the root tip were chosen. These were then drawn with the camera lucida, using oil immersion (Zeiss, 2 mm., N. A. 1.4) and a 20 hyperplane ocular. The drawings were then enlarged 7.25 times with the reflectoscope, giving a total magnification of approximately 22,000 diameters. The area of the chromosomes was then measured with a planimeter, and the length with a pair of dividers set at 0.5 or 1.0 cm. depending on the curve of the chromosome.

In conjunction with these measurements, the lengths and areas of two of the flattest equatorial plates (*A* and *B*) were drawn to scale in figures 4*a* and 4*b*, plate *A* representing figure 1*a*. In figure 4*a* the chromosomes are arranged arbitrarily in their respective groups according to length. Since only two satellited chromosomes were evident in plate *A*, and three in plate *B*, the third being doubtful, the remainder are included in group 3 from which they are indistinguishable on the basis of length. In figure 4*b*, the order of the chromosomes is the same as in figure 4*a*, but since area rather than length is plotted there are individual irregularities referable to the fact that, according to the method used, the chromosome of greatest length may not always be that of greatest area. The general effect obtained from a comparison of figures 4*a* and 4*b* is that the apparent differences in length

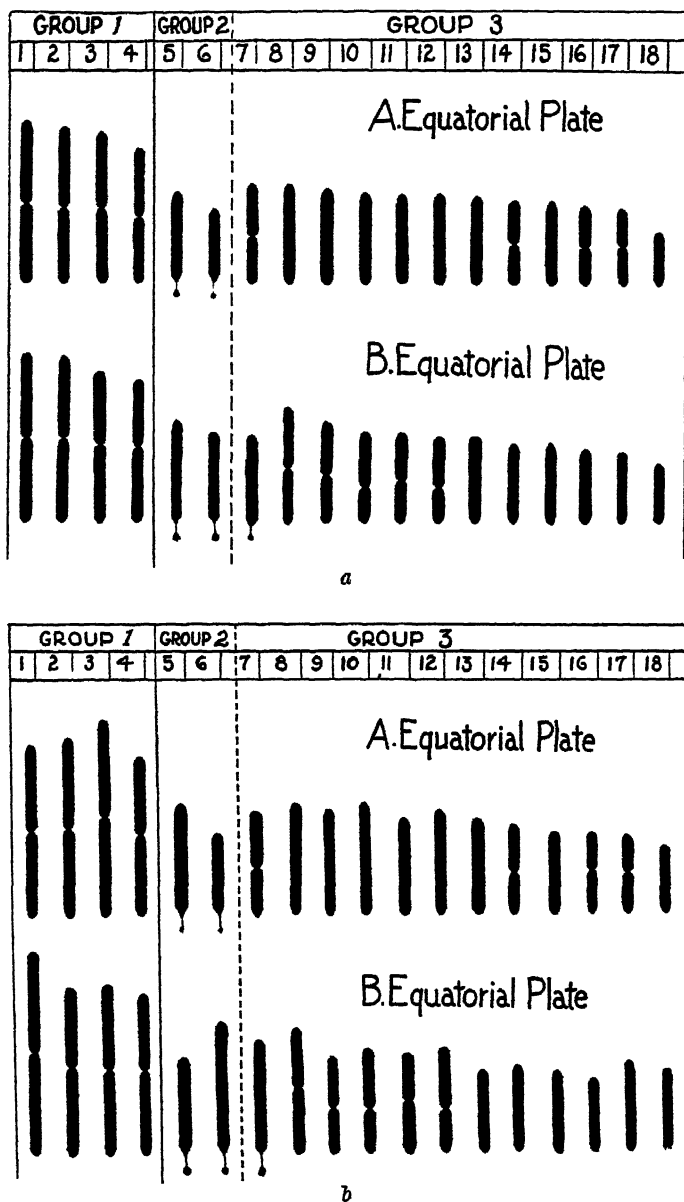


Fig. 4. *N. alata* var. *grandiflora*, chromosomes of two equatorial plates drawn to scale of length in *a*, and area in *b*. Within groups 1, 2, and 3 in *a* the chromosomes are arranged according to length. In *b* the order of the chromosomes is the same as in *a*. Only median constrictions are shown. $\times 3300$.

between the chromosomes within group 1 and within groups 2 and 3 is of little significance. This is further borne out by microscopic evidence which indicates that the apparent difference in length between the four long chromosomes within group 1 and the fourteen medium sized chromosomes within groups 2 and 3 is caused primarily by a foreshortening which is due to the orientation of the chromosomes in the field of the microscope. However, there actually may be a small, discrete difference in length between the chromosome pairs in their respective groups. This actual difference, if present, is not measurable, since the mechanical error due to foreshortening, enlargement, etc., which tend to make such measurements unreliable, is greater than this difference.

The chromosomes of group 3 differ considerably in their positions on the spindle, location of constrictions, and fiber attachments. In every polar metaphase examined, some chromosomes formed V's, others were lying straight, and still others were tilted on end or turned at an angle. It is clear, however, that these positions are not entirely constant for any chromosome. The chromosomes lying in V-shaped positions often appeared to have median constrictions, others appeared to have submedian or subterminal constrictions. There were always some chromosomes in each plate in which no constrictions could be definitely located. Spindle fiber attachment, as observed from a side view of the spindle, appeared to be median or submedian, terminal or subterminal. It was not possible to be certain which chromosome possessed any particular attachment. As shown in figure 1, subterminal as well as median constrictions were quite clear in the case of a few chromosomes. The median constrictions are more clear in all cases and are the only type included in figures 4a and 4b.

These data, on the occurrence and position of constrictions, confirm the conclusions arrived at above as to chromosome groupings according to length. Thus, for example, in figure 4a and b the four chromosomes with median constrictions in plate B are 7, 9, 10, and 11, while in plate A chromosomes 7, 14, 16, and 17 show the constrictions. It seems probable that further investigation of constrictions and fiber attachments in group 3 may make possible its subdivision. The transverse clear areas, already referred to and figured on a medium sized chromosome in figure 3c, B, may be found to be of assistance in this respect.

CHROMOSOME NUMBER AND MORPHOLOGY IN
PMC AND EMC

Chromosome counts were made at diakinesis, heterotypic metaphase and anaphase (IM and IA), telophase (IT), and homotypic metaphase and anaphase (IIM and IIA) from PMC, in both paraffin sections and aceto-carmin smears, and from paraffin sections of EMC. At all stages the haploid number is unmistakably nine, as is shown in what follows.

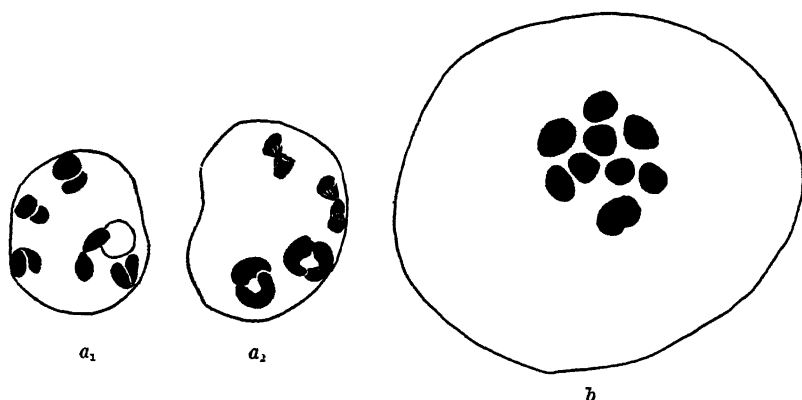


Fig. 5. *N. alata* var. *grandiflora*. a_1 , a_2 , diakinesis from EMC, a_1 showing five bivalent chromosomes and a faintly staining nucleolus; a_2 the remaining four bivalents, two of them being large and ring-shaped. b , IM from PMC, polar view, nine chromosomes, slight size-distinctions. $\times 2300$.

Thirty PMC and EMC were counted in diakinesis and all, with the possible exception of one, showed nine bivalent chromosomes. A typical example of this stage is shown in figure 5 a_1 , a_2 . Here all the chromosomes appear to be about the same size and shape with the exception of the two bivalents in portion a_2 which form rings and appear larger than the others. One, two, or three, usually two, relatively larger ring-shaped bivalents were often seen at this stage. It seems probable that these two large bivalents correspond to the two pairs of large chromosomes always distinguishable in somatic cells. Since the time of contraction may vary for different chromosomes, and the two large bivalents were not always observed, it is difficult to know what weight to give to the evidence as to size distinction at diakinesis.

Sixty counts of PMC at IM were made. In all of these, with three exceptions, nine bivalent chromosomes were counted. They were arranged with considerable regularity on the spindle and were very similar in size and shape, although, just as at diakinesis, two of them often appeared somewhat larger than the others. Figure 5*b* shows the slight size distinctions which are often evident at this stage. Fifteen counts were made at early anaphase and in each of them there were nine bivalent chromosomes undergoing disjunction.

In three cases at IM and in one case at diakinesis, eight bivalents instead of nine were counted. In each of these, however, there was one particularly large chromosome which had a double appearance. This may have represented two bivalents which had fused to form a tetravalent, or what seems much more probable, two bivalents which were temporarily adhering.

Some study was made of IT and interkinesis. Where the chromosomes could be counted there were nine, each of which presented a double appearance, indicating that the longitudinal split for the homotypic division had occurred.

The largest number of chromosome counts was made at IIM in PMC. In determining the chromosome number at this stage, counts were made of all the countable PMC in every field examined. A record was kept of single and double plate counts. As shown in table 1, two types of distribution were commonly found to occur— $\frac{9}{9}$, as would be expected, and less often $\frac{8}{10}$. In addition, chromosomes which had not become included in either nuclei were sometimes seen in the plasma.

TABLE 1
CHROMOSOME NUMBER AT IIM, PMC

Type of distribution.....	$\frac{9}{9}$	$\frac{8}{10}$
Single counts.....	$\frac{397}{0}$	$\frac{16}{25}$
Double counts.....	$\frac{89}{89}$	$\frac{8}{8}$
Total	575	57 or approx. 9%

The proportion of $\frac{8}{10}$ to $\frac{9}{9}$ distribution is comparatively high as indicated by the data in table 1. This proportion is, moreover, variable, being as high as 20 per cent in PMC from old plants bloom-

ing in late fall and as low as 7 per cent from the same plants which having overwintered, were flowering abundantly in the spring. Later counts from plants growing in the field gave a proportion of 3 per cent. The typical $\frac{9}{9}$ distribution at IIM is shown in figure 7a and the $\frac{8}{10}$ distribution in figure 7b.

The considerable proportion of obviously non-disjunctional distribution suggested that *alata* should be exceptionally favorable material in which, at IA and IT, to study the non-disjunctional process. Disjunction was studied at IM and early IA and its results at IIM and

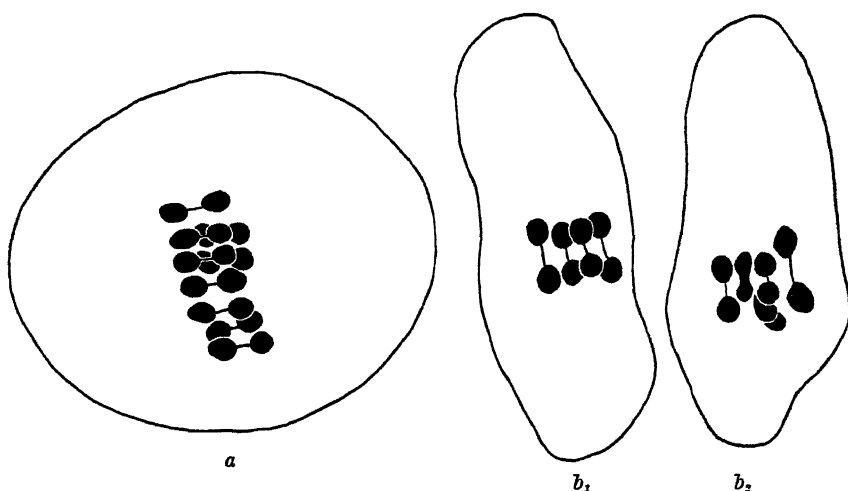


Fig. 6. *N. alata* var. *grandiflora*, early IA, side view. *a*, from PMC, showing nine bivalents disjoining simultaneously. *b₁*, *b₂*, from EMC, showing nine bivalents in one of which disjunction is more advanced and in another less advanced. $\times 2300$.

IIA. The late IM and early IA disjunction of bivalents often appeared to be simultaneous (fig. 6a). Sometimes, however, one bivalent showed a tendency to disjoin somewhat earlier than the others, and another somewhat later. There is no evidence to suggest that these bivalents are the same each time. The earlier disjunction was seen in late IM counts of polar views, where ten chromosomes, rather than nine, could often be counted. The side view of the early IA in figures 6b₁, b₂ from EMC, shows one of the disjoining bivalents slightly more separated than the others. Figure 6b₂ also shows the somewhat later disjunction of one bivalent chromosome. This occurs not uncommonly and is again shown in figure 8a, a slightly oblique polar view from PMC, in which all but one bivalent are disjoining.

Although representing a IIM stage, the condition shown in figure 8b is of interest in this general connection. Here there are eight chromosomes in each plate and two chromosomes very loosely attached near the lower plate. This situation was observed occasionally and apparently is the result of lagging at IA on the part of a non-disjunctional pair. Presumably this pair did not reach a pole in time to be included in a daughter nucleus, but approached one pole so closely as to be incorporated in the corresponding IIM plate.

It is probable that the relatively high percentage of $\frac{8}{10}$ at IIM is a reflection of the frequency with which one bivalent chromosome

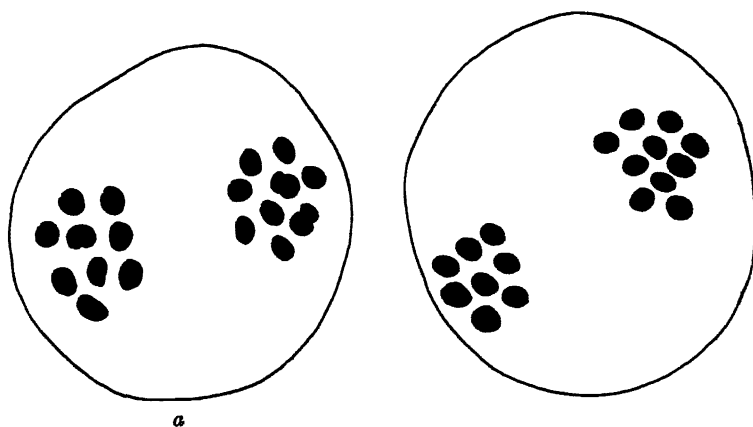


Fig. 7. *N. alata* var. *grandiflora*. PMC, polar view, IIM. *a*, nine chromosomes in each plate. Orientation of two chromosomes on spindle such that split for next division can be seen. *b*, eight chromosomes in one plate and ten in the other. $\times 2300$.

tends to disjoin somewhat later than the others, as was mentioned above. Evidence of non-disjunction was also found in EMC but not enough counts were made to determine the extent to which it occurs. That the $\frac{8}{10}$ distributions observed at IIM in PMC are significant is shown by counts at late IIA of two plates of 10 and two of 8 chromosomes. In other words, it seems clear that considerable numbers of $\frac{8}{10} \mid \frac{8}{10}$ tetrads are produced.

It is also possible that the non-disjunctional chromosomes, either before or after a delayed separation, may lag and at interkinesis lie free in the plasma. In consequence of this it is to be expected that at IIM, $\frac{8}{8} + 1$ or 2, and $\frac{8}{9} + 1$ would be obtained. One or two chromo-

somes were sometimes seen lying free in the cytoplasm at IIM but in these cases it was not possible to obtain counts of both plates. To obtain further evidence in regard to the occurrence of lagging chromosomes tetrad counts were made from four buds from each of fourteen different plants. The results are entered in table 2.

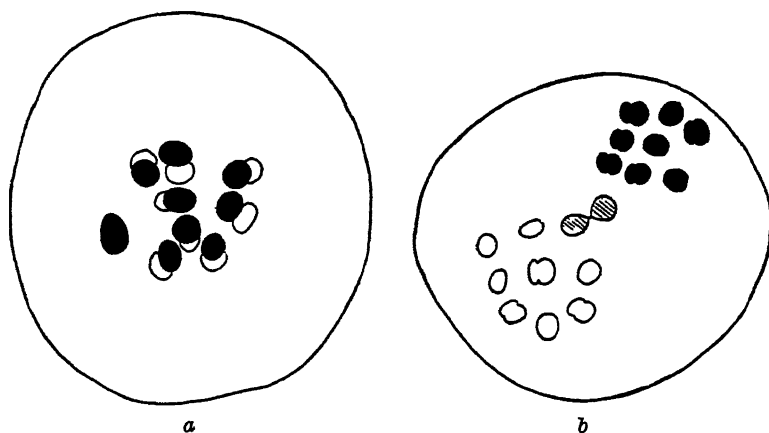


Fig. 8. *N. alata* var. *grandiflora*. PMC. *a*, IA slightly oblique polar view, all chromosomes but one disjoined. *b*, IIM, polar view, eight chromosomes in each plate and one disjoined bivalent incorporated in lower plate (cf. comment in text, p. 171). $\times 2300$.

TABLE 2

COUNTS OF NUMBER OF CELLS AT TETRAD IN FOURTEEN PLANTS OF <i>Alata</i>											
26G065	1	1+1	2	2+1	3	3+1	4	4+1	4+2	4+3	%
P1	0	0	0	0	0	0	720	2	0	0	0.28
P3	0	0	0	0	0	1(?)	782	12	1	0	1.8
P4	0	0	0	0	0	0	797	3	0	0	0.4
P5	0	0	1	0	0	0	392	7	0	0	2.0
P6	0	0	0	0	0	0	787	10	3	0	1.6
P7	0	0	0	0	3(?)	0	1349	35	2	2	3.0
P9	0	0	1	0	0	3(?)	785	5	5	0	1.8
P10	0	0	0	0	0	0	1000	5	0	0	0.5
P15	1	0	0	0	0	1(?)	592	5	1	0	1.3
P41	0	0	36	17	0	0	1273	6	3	0	5.0
P52	0	1	21	4	0	0	1393	53	13	0	6.6
P53	0	0	0	0	0	0	901	3	0	0	0.3
P54	0	0	1	1	0	0	977	32	0	0	3.5
P76	0	0	0	1	0	0	788	11	1	0	1.6

These tetrad counts indicate that, while in the great majority of cases the two meiotic divisions are normal, irregularities do occur. The presence of microcytes (2 + 1, 4 + 1, + 2, + 3) indicates chromosome lagging and failure of inclusion in some cases of such laggards in the

daughter and granddaughter nuclei. Careful search was made for the presence of chromosomes within the cell wall but not incorporated in the nucleus (micronuclei) but they were noted in two cases only. The proportion of irregularities varied from plant to plant, and from bud to bud. This variation from bud to bud was particularly marked in the case of plants 41 and 52, table 2, in which the proportion of dyads was very high in one or two anthers and not in others. The dyads are probably the result of a "semiheterotypic" division (Rosenberg, 1926; Chipman and Goodspeed, 1927, p. 148). The 2-1 case can be explained on the basis of this same type of division plus non-disjunction; the tetrads, and tetrads plus 1, 2, or 3 microcytes, indicate that the normal divisions occurred but that the non-disjunctional chromosomes were not included in the polar groups but were left lying free in the plasma. The proportion of these irregularities as shown in table 2 approximates what might be expected on the basis of the high percentage of non-disjunction noted in table 1.

DISCUSSION

Many observers have found that somatic and meiotic chromosomes, particularly in the second division, can be distinguished on the basis of their length, the position of the constrictions and spindle fiber attachments, and the presence or absence of satellites. Such morphological peculiarities have been shown to be of constant occurrence and have been accepted as distinguishing characteristics. In *alata*, morphological distinctions other than slight size distinctions could not be observed in the meiotic divisions since the chromosomes at both divisions are small, round bodies. In the somatic cells, on the other hand, the eighteen chromosomes could be divided into three groups on the basis of distinct differences in chromosome morphology.

Christow (1925) figures one somatic metaphase of *alata* with sixteen chromosomes. He describes the chromosomes as ". . . lang bogenartig, und leicht zählbar." Since the somatic plate he figures is from ovary tissue, the chromosomes would not be expected to be as distinct and well separated as they are in root tips. One exceptionally long chromosome, for which no homologue is evident, is quite probably two chromosomes and the eighteenth chromosome may have been above or below. No attempt was made by Christow to group the chromosomes, although it is possible in his figure to pick out the four long

chromosomes which have been described in this paper. Greater differentiation might have been obtained had root tips been used. The difficulty apparently experienced by Christow in obtaining division figures in root tips was not encountered by us.

Satellites were figured by Nawaschin (1912, p. 379) on one pair of chromosomes of *Galtonia candicans*. Since then their presence has been reported in various genera by Nawaschin (1925), Taylor (1925a, b, 1926), Langlet (1927), and other observers. Although often hidden, they are unquestionably quite characteristic of at least one pair and probably of two pair of the eighteen chromosomes in *alata*.

A study of meiotic stages in *alata* has revealed the occurrence of non-disjunction to an extent apparently never before reported in the case of plants grown under normal conditions. Stein (1926), however, reports a higher percentage of occurrence in *Antirrhinum* grown from seed subjected to radium emanations. In *alata*, counts at IM leave no room for doubt that, as indicated by counts of eighteen in the somatic cells, the characteristic haploid number is nine. On the other hand, counts at IIM show that $\frac{8}{10}$ rather than $\frac{9}{9}$ distributions occur in from 3 to 20 per cent of the PMC studied. In passing, it may be noted that PMC from late season plants of *N. Sanderae* show a still higher percentage of $\frac{8}{10}$ distributions. The high percentage of non-disjunction answers the question raised by Goodspeed (1923) as to whether the chromosome number of *alata* is 8, 9, or 10 pairs. Indeed he figures an $\frac{8}{10}$ distribution at IIA. Christow's (1925) report of eight as the haploid number may in part depend upon similar homotypic counts and also upon his idea, ". . . dass *N. tabacum* triploid von *N. alata* ist"

As yet no evidence has been obtained that non-disjunction, while occurring at least occasionally, is equally characteristic of IA in EMC. Unless, however, 8- and 10-chromosomed pollen grains are uniformly non-viable, it would appear that in *alata* monosomic and trisomic individuals should occur. With this point in mind extensive cultures of this species are under investigation and controlled pollination is being employed on the supposition that *alata* pollen tubes of abnormal chromosome constitution may suffer in competition with those of normal chromosome constitution. One plant has already been found whose chromosome complement is $2n-1(17)$.

In connection with the data submitted above, it might be pointed out that *N. longiflora* apparently possesses ten pairs of chromosomes and in external morphology is not too distinctly set apart from *N. alata* var. *grandiflora*. At IM of F_1 hybrids between the two species $9_{II} + 1_I$ normally occur but complete sterility appears to be the rule. A comparative study is being made of chromosome morphology in *longiflora* and in the F_1 with *alata*, in conjunction with further studies of *alata* itself, which it is hoped will give more evidence on some of the matters referred to above.

SUMMARY

1. The somatic chromosomes of *Nicotiana alata* var. *grandiflora* are eighteen in number and can be separated into three groups on the basis of chromosome morphology.

2. In the first group are two pairs of long chromosomes with median constrictions, in the second is at least one pair, and probably two pairs of medium sized chromosomes bearing proximal satellites, and in the third are five or six pairs of medium sized chromosomes indistinguishable from one another except, possibly, on the basis of location of constrictions.

3. The haploid chromosome number is nine, but as a result of non-disjunction from 3 to 20 per cent of IIM in PMC may show $\frac{8}{10}$ rather than $\frac{9}{9}$.

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INTERSPECIFIC HYBRIDIZATION IN NICOTIANA

VII. THE CYTOLOGY OF HYBRIDS OF THE SYNTHETIC
SPECIES, DIGLUTA, WITH ITS PARENTS,
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INTRODUCTION

Within recent years a number of instances have been described of the production of interspecific hybrids with the sum of the somatic, not the gametic, chromosome numbers of the parents. The classical instance is that of *Primula kewensis* (18_n), which arose from hybridization of *P. floribunda* (9_n) with *P. verticillata* (9_n); others are the tetraploid interspecific tobacco hybrid, called *Nicotiana digluta* (36_n) in this paper, which arose from hybridization of *N. glutinosa* (12_n)

¹ The cytological studies reported herein were conducted in the Botanical Institute of the University of Stockholm. The author is deeply indebted to Professor Otto Rosenberg, not only for the provision of facilities, but also for his helpful interest in the investigations. The cultures reported upon were grown at the University of California. The author is indebted for preparation of acetocarmine smears to Miss Mabel L. Ruttle, Miss Edna L. Hollingshead, and Mr. J. M. Webber.

with *N. tabacum* (24_n); the *Aegilotriticum* (28_n) hybrids of Tschermak and Bleier (1926), which arose from crosses of *Aegilops taeta* (14_n) with varieties of *Triticum durum* (14_n) and *T. dicoccoides* (14_n); and the tetraploid *Raphanus-Brassica* hybrids (18_n) discovered by Karpechenko (1927) in the F₂ of a cross of *Raphanus sativus* (9_n) with *Brassica oleracea* (9_n). *Rosa Wilsoni* (21_n), a hybrid of *R. pimpinellifolia* (14_n) with *R. tomentosa* (7_n + 21_n), described by Blackburn and Harrison (1924) belongs to the same category when proper allowance is made for the mode of gamete formation known to occur in its male parent. Besides these instances among seed plants the analogous results secured by F. v. Wettstein (reviewed by ...) by applying the regeneration methods of the Marcha to moss hybrids may be mentioned. Increase in chromosome number following hybridization has also been observed in other instances; but those mentioned specifically constitute a distinct category manifested from the time of their origin by a relatively high degree of fertility and constancy, a consequence of their balanced chromosomal constitution. This phenomenon is of particular importance to economic practice for it opens a way to the utilization of interspecific hybrids not hitherto of value because of their sterility or inconstancy. It is also of importance to evolutionary theory since it demonstrates a method of permanent increase in chromosome number not open to some of the objections urged against variation in chromosome number within a species as a point of departure.

A previous paper (Clausen and Goodspeed, 1925) contained the preliminary account of the production of a constant, fertile form from a cross between *Nicotiana glutinosa* and *N. tabacum*, and it was shown that it had arisen through doubling of the chromosome number normal to F₁. The new form thus obtained has remained constant for five generations; and, although an uncertain seeder, it has a rather high degree of fertility under favorable conditions. Aside from its fertility, it bears the same relation to normal F₁ that gigas forms in general bear to their normal progenitors.

This form cannot be considered a variety of either *glutinosa* or *tabacum* inasmuch as, considered solely from a morphological point of view, it exhibits distinctive features not shown by either parent. These may be appreciated by reference to figure 1, which illustrates flower form features of the two parent species, the normal F₁ and the fertile gigas form. The last is obviously merely an enlarged expression of normal F₁; its abundant pollen, as contrasted with the scantiness of that of normal F₁, testifies to its fertility. In general form the

flower of the hybrid exhibits effects derived from both parents. Its large, deeply incised calyx, its ample, flaring, infundibulum, and its distinct laterality are reminiscent of *glutinosa*: its broad, pentagonal, richly-colored limb is more like that of *tabacum*; while its strongly exserted pistil and stamens represent a new feature not seen in either parent but very strikingly exhibited in another species of *Nicotiana*, the tree tobacco, *tomentosa*. Compared with either parent alone, therefore, it exhibits distinctive morphological differences as great as those marking many recognized species and of the same

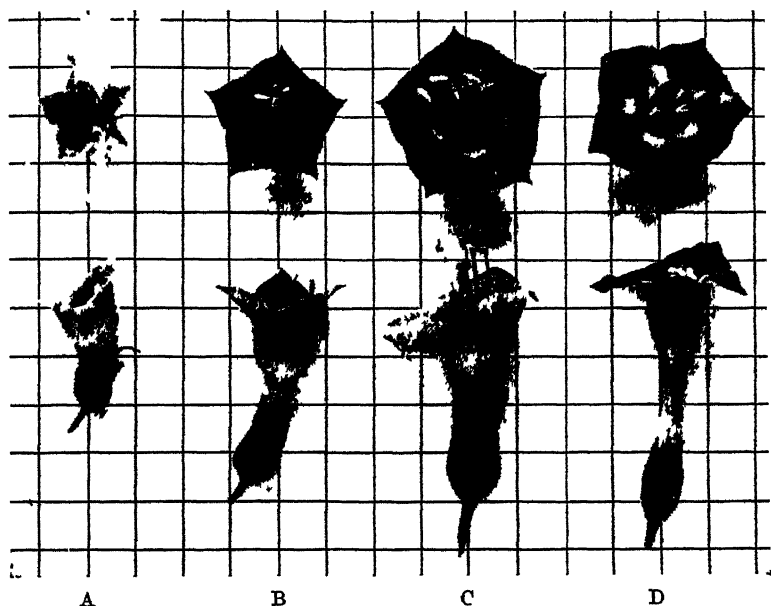


Fig. 1. A, *N. glutinosa* (24_{GG}); B, normal F₁ *glutinosa-tabacum* (12_G + 24_T); C, *N. digluta* (12_{GG} + 24_{TT}); D, *N. tabacum* (24_{TT}). Background ruled in cm. squares. Note that *N. digluta* is simply an enlarged expression of normal F₁ *glutinosa-tabacum*, aside from fertility, which is evidenced by abundant pollen production.

general character; i.e., numerous, comparatively minute differences affecting all portions of the individual rather than few, comparatively conspicuous differences confined to restricted features. Compared with both parents, however, it is readily seen that, aside from the exsertion of pistil and stamens, these morphological features represent a synthesis of those of the two parents. Similarly in habit of growth, leaf characters, and inflorescence, influences of both parents are seen, although the ensemble gives the general impression of closer approach to *tabacum* than to *glutinosa*. Because of its morphological

and cytological features and its behavior in crosses with the parental species, described subsequently in this paper, it may be considered a distinct species. For convenience we shall henceforth call it *Nicotiana digluta* (didiploid *glutinosa-tabacum*), an acrostic abbreviation descriptive of its cytological and genetic constitution.

Some confusion has arisen in the literature as to the manner in which *digluta* was produced; but we believe that the circumstances of its origin are sufficiently clear to leave no doubt on this point. The present line arose from an F_1 plant described as partially fertile. It was late in maturing and had to be transferred to the greenhouse in order to secure seed from it. On account of poor conditions it did not live long, but by selfing several capsules were obtained from it, some of which contained as many as 200 to 300 viable seeds. This plant was not studied cytologically; but its progeny, consisting of 65 plants which may be designated F_2 , was uniform except for one very large plant; and the five plants from which seed was gathered all exhibited $36n$ chromosomes at I-M. The lack of uniformity in size has also characterized later populations and has been shown to have no genetic significance; and the fertility of *digluta* as now grown is no greater than that of the original F_1 plant. It was therefore concluded that the F_1 plant itself must have possessed the double number of chromosomes.

This conclusion has been supported by all subsequent evidence. Every year since the discovery of *digluta*, populations of F_1 *glutinosa-tabacum* have been grown and studied in various ways. They have always been uniformly of the normal F_1 type, and none of them has ever produced any viable seed either from flowers blooming in the open or from those pollinated under bag with pollen of *glutinosa* or *tabacum*. It may be possible that with more extensive trials a normal F_1 plant may produce a few seeds after the method described by Karpechenko (1927) for F_1 Raphanus-Brassica hybrids, but as yet none has been secured; and it would still be quite impossible to explain the original, comparatively highly fertile plant on this basis. There seems to be, therefore, no escape from the conclusion that the original fertile F_1 plant arose from a doubling of the chromosome number in a somatic division of the hybrid zygote, immediately or shortly after hybridization, a method of origin which is in agreement with the observations of Blakeslee, Belling and Farnham (1923) on tetraploid *Datura* and those of Pellew and Durham (1916) on direct production of *Primula kewensis* from crosses of *P. floribunda* and *P. verticillata*.

While the mode of origin of forms such as these is unquestionably a problem of great importance, it is also a matter of interest to ascertain the subsequent phenomena exhibited by them. This paper, therefore, is concerned with a preliminary report on this latter phase of the general problem, specifically with the cytological relations of *digluta* to its parental species, *glutinosa* and *tabacum*, as exhibited in F_1 hybrids, and with the mode of distribution of chromosomes in such hybrids, as evidenced by individual studies of chromosome numbers in their progenies.

It would also have been of advantage to have been able to study more minutely the cytological phenomena in *digluta* and in normal F_1 *glutinosa-tabacum*; but unfortunately the available material was not satisfactory for the purpose. *Digluta* certainly exhibits some irregularities in chromosomal distribution as is evidenced by a certain amount of lagging in meiosis and by the production of microcytes in pollen groups, in which respect it resembles the *Aegilotricum* hybrids of Tschermak and Bleier (1926). Normal F_1 *glutinosa-tabacum* also deserves further attention. The earlier report that it exhibited conjugation according to the Drosera scheme appears to have been in error, for later examination indicates weak affinity of the *Hieracium boreale* type (Rosenberg, 1917), so that usually the number of pairs of chromosomes is less than twelve. Evidently *glutinosa* chromosomes have much weaker affinity for *tabacum* chromosomes than those of *sylvestris* and *tomentosa*, which regularly exhibit $12_{II} + 12_I$ at I-M in their hybrids with *tabacum*. However, inasmuch as the available material did not permit a quantitative study of these matters, they are reserved for future treatment.

METHODS

The studies reported herewith were conducted under some difficulty owing to the fact that paraffin material proved to be unreliable. For this reason most of the work was done on aceto-carmin smears of PMC, most of which were prepared in Berkeley, California, and shipped to Stockholm, Sweden, where the examinations were made. For the most part, the preparations arrived in perfect condition and remained good for as long as two or three months after their arrival. They were sealed with a wax preparation consisting of equal parts of paraffin and gum mastic, which permitted repeated studies of the same preparation under oil without serious displacement due to cleaning. While such aceto-carmin smears are much less intensely stained

than iron-haematoxylin preparations, they are perfectly satisfactory for stages from I-M on, and in some instances they give good figures of earlier stages. In a few cases, satisfactory iron-haematoxylin preparations were obtained and checked against the aceto-carmin smears, and the results were found to be in perfect agreement.

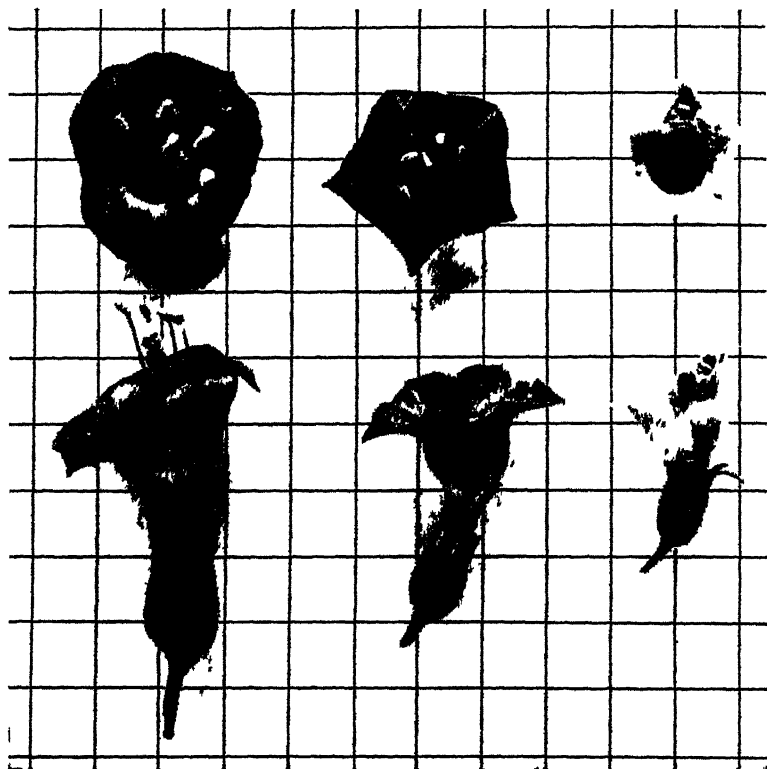


Fig. 2. A, *N. digluta* ($12_{GG} + 24_{TT}$); B, F_1 *digluta-glutinosa* ($12_{GG} + 24_T$); C, *N. glutinosa* (12_{GG}). Background ruled in cm. squares. Note that F_1 is intermediate in size and general characteristics. Its sterility is evidenced by the scanty pollen production.

Drawings of chromosome garnitures were made with the aid of a camera lucida; a combination of a 1/12 oil immersion lens and a $\times 20$ applanatic eyepiece giving, at table level, figures magnified about 3400 diameters, which were reduced to $\times 2000$ in reproduction.

In certain respects the drawings are semidiagrammatic. No attempt has been made to represent differential staining within the chromosomes, although it was often conspicuous. The outlines of the

chromosomes were drawn individually at their maximum extent, and filled in to give silhouette figures. Inasmuch as univalent chromosomes stained more lightly than bivalents, the former have been represented in gray, the latter in black. Overlapping has been treated in various ways, as circumstances required, but no attempt has been made to show by differential shading the relative levels at which the chromosomes lay.

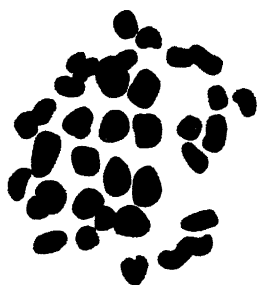


Fig. 3.

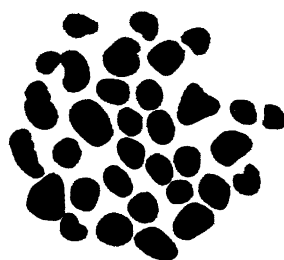


Fig. 4.

Figs. 3 and 4. I-M, polar views, univalent chromosomes in gray.

3, F_1 *digluta-glutinosa*, $12_{CG} + 24_T$. 4, F_1 *digluta-tabacum*, $24_{TT} + 12_{CG}$.

F_1 *digluta-glutinosa*

Digluta crosses readily with *glutinosa* when the former is used as the female parent, but the reciprocal cross has hitherto been unsuccessful. The hybrid is vigorous, and intermediate in general features between *digluta* and *glutinosa*. In figure 2 it may be seen that the size of the flower and its general appearance lie between those of the two parents. The marked exsertion of pistil and stamens has disappeared. A conspicuous feature of the hybrid is its high degree of sterility. No progeny has yet been obtained from it even in backcrosses to the parents; but it does produce a few seeds and doubtless some of these will prove viable under more extensive trials. As compared with hybrids between recognized species, F_1 *digluta-glutinosa* is probably more highly sterile than F_1 *paniculata-rustica*, but less so than F_1 *sylvestris-tabacum*.

Some features of F_1 cytology are represented in the accompanying figures. At I-M in polar view (fig. 3) 12 bivalent and 24 univalent



Fig. 5.



Fig. 6.

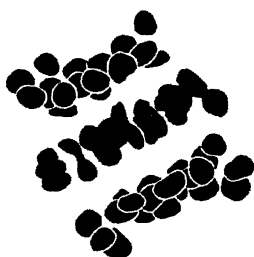


Fig. 7.

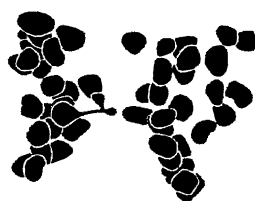


Fig. 8.



Fig. 9.



Fig. 10.

chromosomes were easily detected. Differential staining of the univalents and bivalents was very distinct. The univalents were usually arranged peripherally around the equatorial plate, although rarely in exactly the same plane, and even when above or below the plate they seemed to lie on the surface of the spindle figure rather than in it. In side view they may take up a very scattered arrangement, as shown in figure 6, or they may be rather closely aggregated in the region of the equatorial plate, as illustrated in figure 5; but more often the arrangement is intermediate between these extremes. It seems that the arrangement at this stage may have an effect upon the anaphase figure. Thus a metaphase of the type shown in figure 6 apparently gives rise to an anaphase such as is illustrated in figure 8, in which the bivalents separate and the univalents are distributed at random to the two poles. If the metaphase is of the type shown in figure 5, however, the univalents at the equator apparently remain there after the bivalents disjoin (fig. 7), and under such circumstances some of them may divide. Usually, however, both members of such dividing univalents pass to the same pole without completing the division or, if completed, half of the chromosome may remain in the plasma and the other half may become incorporated in one of the daughter nuclei. There is a great deal of irregularity in this division as is particularly well evidenced by interkineses, which often show several micronuclei, representing either undivided univalents or halves of divided ones, in addition to the large daughter nuclei. In figure 8 the distribution is $23 < > 25$; in figure 9, neglecting the small chromatin body which has evidently arisen by fragmentation, it is $24 < > 24$; and in figure 10, representing a particularly well spread anaphase in a large cell, it is $20 < > 28$. The univalents in the first division, therefore, appear to be for the most part distributed at random undivided; but a small amount of division of univalents and a rather large amount of lagging occurs, leading to the failure of some univalents or halves thereof to be incorporated in the daughter nuclei.

Figs. 5-10. *F₁ digluta-glutinosa*, $12_{aa} + 24_r$, illustrating various stages of meiosis, all chromosomes represented in every figure. 5, I-M, side view, univalents closely grouped around equatorial plate. 6, I-M, side view, univalents widely scattered. 7, I-A, side view, 12 univalents lagging, some in process of division; 16 chromosomes in upper group, 20 in lower. Presumed to have arisen from a I-M arrangement as in figure 5. 8, I-A, side view, 23 chromosomes in left group, 25 in right, no division of univalents. Presumed to have arisen from a I-M arrangement as in figure 6. 9, I-A, side view, 24 chromosomes in each group, no division of univalents. Note fragmentation of one of the chromosomes. 10, late I-A, unusually well spread, in a large cell; 20 chromosomes in one group, 28 in the other, no division of univalents.

No figures of second divisions are offered since they did not differ essentially from those of *F₁ digluta-tabacum*, which are illustrated. Lagging was also observed in II-A, which appears to be a consequence, judged by the size of the laggards and their behavior, of division of univalents in I. The lagging led to a further increase in the number of excluded chromosomes, so that with final conclusion of the divisions, four large microspore nuclei and several very small micronuclei were usually present in the PMC. Further comments as to chromosome elimination are contained in a separate section below.

F₁ digluta-tabacum

Digluta also crosses readily with *tabacum* when the former is used as the female parent but hitherto, as in the previous case, the reciprocal hybrid has not been obtained. Such crosses give a uniform progeny, at first glance rather closely resembling *tabacum* but on closer examination exhibiting an intermediate expression of characters throughout. This is well shown in the flower features of the three types portrayed in figure 11. Observe particularly the reduction in the size of the infundibulum and the less pronounced exertion of pistil and stamens, which, however, is still rather striking.

In contrast to *F₁ digluta-glutinosa*, *F₁ digluta-tabacum* is highly fertile, to such an extent that the capsules often appear to be filled with seeds. It seems to produce seed of almost equal quantity either from self-fertilization or from backcrosses to *tabacum*. The order of fertility is far higher than that which we have observed in any proper interspecific hybrid in *Nicotiana*. Although there is some chaffy material, most of the seeds are viable and give rise to vigorous seedlings.

Cytological features of *F₁ digluta-tabacum* are closely comparable with those of *F₁ digluta-glutinosa*, with proper allowance for the difference in chromosome number. In I-M there are 24 bivalents and 12 univalents, as shown in figure 4. Sometimes all twelve univalents may be detected in side views, but more often some of them lie scattered around the periphery of the equatorial plate. In I-A the univalents may be distributed without conspicuous lagging; but more often there are a number of laggards, and in extreme cases, as in figure 12, all the singles may be detected. In this figure the twenty-four bivalent partners are at the poles, eleven of the univalents are lagging and one apparently undivided univalent lies in an unusual

position at the periphery of the cell. The figure shows, however, that only a few of the univalents are in a position to complete distribution of their halves to the two poles of the spindle. In most of them, even though the division is well advanced, both halves are evidently going together to the same pole. An unusual, late anaphase in a very large

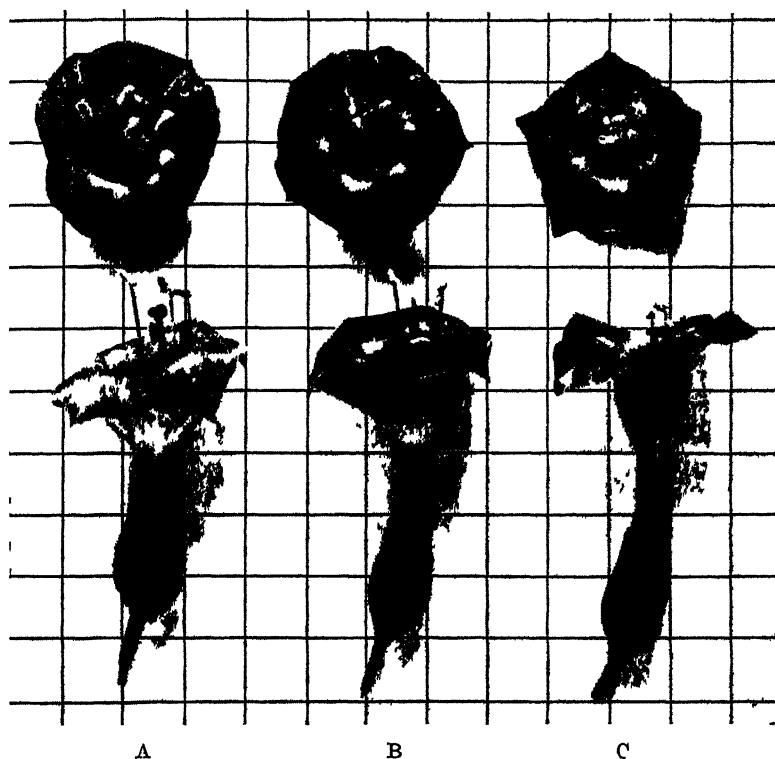


Fig. 11. A, *N. digluta* ($12_{GL} + 24_{RT}$); B, F_1 *digluta-tabacum* ($24_{RT} + 12_{GL}$); C, *N. tabacum* (24_{RT}). Background ruled in cm. squares. Note intermediacy of characters of F_1 .

cell, represented in figure 13, apparently illustrates the usual fate of such attempts at division of the univalents. The distribution is here $28 < > 32$, but both groups contain univalents in advanced stages of division with the halves, however, still connected by slender strands. As in F_1 *digluta-glutinosa* there is often a considerable amount of chromosome elimination at this point.

A typical II-M figure, which indicates that some division must have occurred in I, is illustrated in figure 14. Here the distribution is $29\frac{1}{2} + 1\frac{1}{2} + 28\frac{3}{2}$. Apparently four undivided univalents went

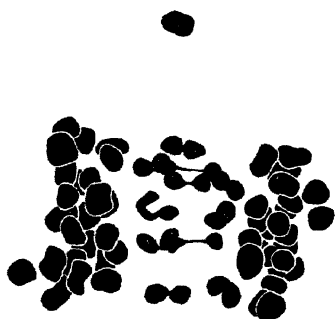


Fig. 12.



Fig. 13.

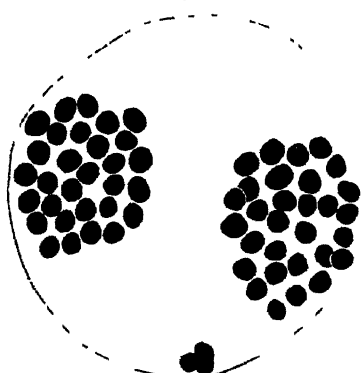


Fig. 14.

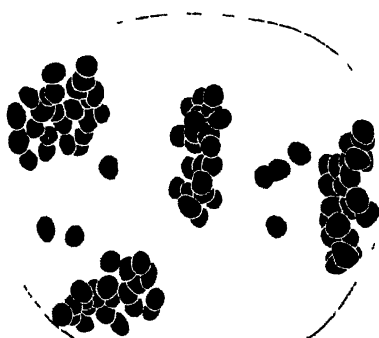


Fig. 15.

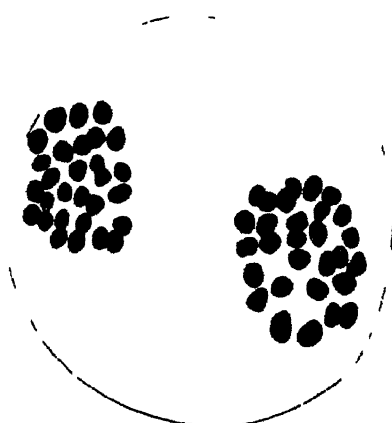


Fig. 16-A.

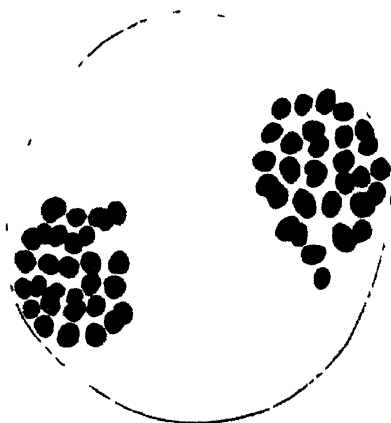


Fig. 16-B.

to one pole, four to the other, one remained in the plasma, one divided in such a way that half of it was included in each daughter nucleus, and one divided in such a way that half of it was included in one daughter nucleus and half remained in the plasma. The half chromosomes in these metaphase plates were conspicuously paler than the others. Further evidence that division sometimes occurs in some univalents in I is afforded by lagging in II-A, a rather extreme instance of which is illustrated in figure 15. There are four laggards in one anaphase, three in the other, and an additional half univalent lying in the plasma above one of the groups. The sizes of these laggards and their failure to show any evidence of division seem sufficient to mark them definitely as halves of univalents; but it was not possible to count the chromosomes in the polar groups as would have been desirable. In a number of instances it was possible to count all four groups of chromosomes in II-A. One such preparation, shown in figure 16, exhibited a distribution of $\frac{29}{31} + \frac{29}{31}$, so that at least two chromosomes must have divided in I. Another was $\frac{29}{32} + \frac{29}{30}$, which would necessitate division of at least three univalents in I. Completion of meiosis usually resulted, as in the previous hybrid, in production of four microspore nuclei and a variable number of micronuclei, representing chromosomes eliminated during the process.

F₁ CHROMOSOMAL RELATIONS

From the above results it is clear that F₁ *digluta-glutinosa* may be represented by the chromosomal formula, $12_{GG} + 24_T$, and F₁ *digluta-tabacum* by $24_{TT} + 12_G$, wherein pairs of *glutinosa* and *tabacum* chromosomes are indicated by *GG* and *TT*, respectively, and uni-

Figs. 12-16. F₁ *digluta-tabacum*, $24_{TT} + 12_G$, illustrating various stages of meiosis, all chromosomes represented except in figure 15. 12, I-A, side view, 11 univalents lagging and in various stages of division, remaining one at periphery of cell; illustrating extreme condition of lagging. 13, late I-A, unusually well spread, in a large cell; 28 chromosomes in left group, 32 in right. Note presence of incompletely divided univalents in each group, presumably illustrating usual fate of attempted division of univalents. 14, II-M, distribution, $29\frac{1}{2} + 1\frac{1}{2} + 28\frac{1}{2}$, half univalents in gray. Two univalents must have completed division in I. 15, II-A, side view, illustrating lagging in II. Not all of chromosomes in polar groups distinguishable. Four laggards in one II-A figure, three in the other, and a fourth above one of the polar groups. Laggards represented in gray merely to distinguish them from the others; actually equivalent in size, staining capacity, and behavior to other chromosomes, hence must have arisen by division of four univalents in I. 16, late II-A; A, upper two groups and B, lower ones in the same cell. Distribution, $\frac{29}{31} + \frac{29}{31}$.

valents by *G* and *T*. This, of course, is precisely what one would expect, if the chromosomal formula of *digluta* is $24_{TT} + 12_{GG}$, as has been assumed.

In both hybrids the bivalent chromosomes behave normally, and the univalents are distributed for the most part at random in I and divide in II; but a few of them may divide in I, whereupon the halves are distributed at random in II. From a genetic standpoint, it is of course immaterial whether the univalents are distributed at random in I and divide in II, or the reverse, as long as they divide only once. As a consequence the gametic series of F_1 *digluta-glutinosa* should be $12_G + n_T$, the value of *n* and the relative frequencies of its different values being determined by expansion of the binomial, $(.5 + .5)^{24}$. Similarly the gametic series of F_1 *digluta-tabacum* should be $24_T + n_G$, with values and proportionate frequencies of *n* given by $(.5 + .5)^{12}$. As will be shown below, however, particularly for the F_1 *digluta-tabacum* series, account must be taken of the effect of chromosomal elimination on these ideal gametic series.

These results are essentially in agreement with those reported by Karpechenko (1927) for comparable plants secured by backcrossing F_1 *Raphanus-Brassica* to *Raphanus*. A large proportion of such plants were found to have 27 chromosomes, consequently they must have had nine pairs of *Raphanus* chromosomes and nine *Brassica* chromosomes. The meiotic phenomena were usually in accord with the formula, $9_{RR} + 9_B$, with the important exception, however, that a small proportion of the reduction divisions apparently passed through an abbreviated cycle, giving restitution nuclei, according to the scheme of Rosenberg (1927), at the close of I and a single, essentially somatic division in II, so that two cells were formed possessing nearly, if not quite, the full somatic complement of chromosomes. These and other irregularities were not observed in the *Nicotiana* material; and if they do occur it must be in a much lower ratio of frequency than in the *Raphanus-Brassica* material, as is indicated by the studies of pollen cell formation reported below.

On the basis of the chromosomal behavior in hybrids, *digluta* may, therefore, be considered a distinct species, because the phenomena exhibited by its hybrids with *glutinosa* and *tabacum* are in all essential respects parallel to those exhibited by other interspecific hybrids in *Nicotiana* which have been found to conform to the *Drosera* scheme; viz., F_1 *paniculata-rustica* (Goodspeed, Clausen, and Chipman, 1926), F_1 *sylvestris-tabacum* (Goodspeed, 1923), and F_1 *tomentosa-tabacum*.

F_1 *digluta-tabacum* \times *tabacum*

Two populations of F_1 *digluta-tabacum*♀ \times *tabacum*♂ were grown, one, G25144, of 80 plants in the greenhouse in the winter of 1925-26, and another, 26187, of 50 plants in the field in 1926. The plants grown in the greenhouse were in twelve-inch pots, which permit development almost equal to that in the field. The populations were each grown from seed of a single capsule; but they only represented a small sample, presumably chosen at random, of the progenies which might have been grown from these capsules.

The progeny grown in the greenhouse was studied rather carefully from a morphological point of view, but with indifferent results. With the exception of two plants all produced flowers, the growth was normal throughout, and the plants were all apparently highly fertile. They exhibited considerable variability, but of a type difficult to describe or classify in any satisfactory fashion. There were minor differences in leaf size and shape, habit of branching, flower size and shape, exertion of pistil and stamens, etc. After chromosome numbers had been determined, it was noted that those having $24_{II} + 0_I$ chromosomes were identical with *tabacum* in morphological features, that those which had $24_{II} + 1_I$ chromosomes differed only slightly from *tabacum*, and that as the number of extra chromosomes increased, the differences both from *tabacum* and within the group became more pronounced. The $24_{II} + 1_I$ plants appeared to exhibit individual features but whether these will prove distinct enough to permit accurate separation from *tabacum* and from one another remains to be seen.

Our greatest concern in these populations was with chromosome numbers of individual plants, inasmuch as the phenotypic segregation was too complicated and the differences too slight to permit a genetical analysis. The material proved to be eminently suited for chromosome counting. I-M stages in polar view were usually well spread and the univalents were readily distinguishable by reason of their paler appearance, smaller size, tendency to assume a kidney shape as a consequence of the onset of division, and peripheral position in relation to the equatorial plate formed by the bivalents. For illustrations of comparable types of chromosome groups, reference may be made to figures 31 to 36, showing conditions in F_2 *digluta-tabacum* segregants, which belonged to the same cytological series.

An examination of the data contained in table 1 reveals the fact that the two populations were in close agreement as to distribution of chromosome numbers. In both populations every plant belonged to the series, $24_{II} + n_I$, with n having values from 0 to 8. In the greenhouse culture the average number of univalent chromosomes per plant was 2.20; in the field-grown progeny, 2.32; and in the two populations combined, 2.25. Inasmuch as the female parent had twelve univalent chromosomes, the stipulations of random distribution would call for an average of six univalents per plant. The results actually obtained, therefore, indicate that only about 40 per cent of the expected number of univalents descends to the progeny in this series. Or stated in another way we may say that the chance, represented by q , of a given univalent's descent to any plant is $2.25 \div 12 = 0.19$, instead of 0.50, the value to be expected with undisturbed random distribution.

TABLE 1

FREQUENCIES OF CHROMOSOME NUMBERS IN PROGENIES OF F_1 *digluta-tabacum* ♀ × *tabacum* ♂. ALL PLANTS BELONGED TO THE SERIES, $24_{II} + n_I$, n HAVING VALUES FROM 0 TO 8

Garden number	Number of univalents									Total
	0	1	2	3	4	5	6	7	8	
G25144	5	14	20	6	8	1	1		1	56
26187	3	11	6	9	6		2			37
Total observed	8	25	26	15	14	1	3		1	93
Calc $93(81 + 12)^{12}$	7.4	20.9	27.0	21.1	11.1	4.2	1.1	0.3	0.0	93.1
Observed per 500	43.0	134.4	139.8	80.7	75.2	5.4	16.1		5.4	500.0
Calc 3000(5 + 5) ¹²	0.7	8.8	48.4	161.1	362.5	580.0	676.7	580.0	362.5	2999.7*

* Including remaining terms

In the interpretation of these results two main causes may be appealed to: (1) selective inviability either of gametes or zygotes having univalent chromosomes, and (2) elimination of univalent chromosomes during meiosis. As to inviability it must be recalled that the two populations represent presumably random selections of larger populations. It is possible that 500 plants could have been grown from these two capsules; therefore a comparison should be made of a population of 500 plants, distributed according to the frequencies of the different chromosome numbers actually observed, with a population representing the maximum possible production of the two capsules, say 3000, distributed purely at random. The results

of the necessary computations are contained in the last two lines of table 1, a comparison of which demonstrates that an appeal to inviability alone is untenable because of the high concentration of individuals in the lower three classes in the observed series. Moreover, the absence of any individuals whatever in the higher classes and the fact that few, if any, of the plants actually grown exhibited any of the disharmonies peculiar to populations in which there is reason to suspect elimination from inviability, also indicate that this factor is a relatively unimportant agency in this instance.

On the other hand the observed frequency of chromosome elimination, concerning which more will be said below, provides direct evidence on the importance of this phenomenon. If there is no elimination from inviability, then the distribution arising from a situation in which chromosomes are eliminated during meiosis will be of the form, $(p + q)^n$, where p represents the proportionate chance of exclusion of a univalent, q of inclusion, and n the number of univalents. For the present case, therefore, observation should agree with the terms obtained by expansion of the expression, $93 (.81 + .19)^{12}$, which is actually the case, as is shown by comparison of observed and calculated distribution series in the table. Further support of this argument is afforded by agreement of the standard deviations of the two series; viz.,

Observed series	$M = 2.25 \pm 0.15$	$\sigma = 1.47 \pm 0.11$
Calculated series	$M = 2.28 \pm 0.13$	$\sigma = 1.36 \pm 0.10$

Here the standard deviation of the observed series has been determined directly from the distribution in the usual fashion; but that for the calculated series has been computed by proper substitution of the values from which it was derived in the general formula,

$$\sigma = \sqrt{n \cdot p \cdot q}.$$

Although the agreement is satisfactory, it cannot, however, be considered positive proof that chromosomal elimination alone is responsible for the observed results.

CHROMOSOMAL ELIMINATION

That chromosomal elimination is an important desideratum in this material is shown very strikingly by direct examination of pollen groups, which often contain, in addition to the four normal microspores, a variable number of comparatively small microcytes. The microcytes usually contain a single, very small nucleus, but occasion-

ally they contain two or more micronuclei, from which fact, together with the small variation in size of the micronuclei, it may be concluded that a micronucleus usually represents a single half-univalent. A typical series of pollen groups of the F_1 *digluta-tabacum* hybrid, illustrative of these features, is shown in figure 17.



Fig. 17. F_1 *digluta-tabacum*, $24_{II} + 12_G$. Pollen groups illustrating microcyte production. Nuclei in broken outline. All cells have four large microspores, with from 0 to 7 microcytes. Note that some microcytes have more than one micronucleus. $\times 1000$.

It is not difficult to make an enumeration of cells in pollen groups in favorable material. Aceto-carminine smears are particularly good because the entire group lies undisturbed in the preparation. Extensive counts of microcyte formation were made in the parent species, in F_1 hybrids and in individual plants of the F_1 *digluta-tabacum* \times *tabacum* backcross progeny having various numbers of univalents. Typical results are recorded in table 2, wherein microcyte formation in this series may be compared with that occurring in other typical interspecific hybrids.

While these data have only a limited value, there are a number of things which stand out rather clearly. The parental species, *glutinosa* and *tabacum*, and the same is also true of other species, produce very few microcytes. Compared with them, the results of meiosis in *digluta*

are obviously much more irregular. The most abundant microcyte formation occurs in F_1 *digluta-glutinosa*, which has the greatest number of univalents; but F_1 *digluta-tabacum* appears to produce a greater number of microcytes than F_1 *sylvestris-tabacum* and F_1 *tomentosa-tabacum*, which have the same number of univalents. It is possible, however, that the counts from the latter two, together with those from F_1 *glutinosa-tabacum*, are not so accurate, since they were made a year earlier when the writer was probably less skilled in detecting microcytes.

TABLE 2

MICROCYTE FREQUENCIES IN VARIOUS SPECIES AND HYBRIDS OF NICOTIANA. UPPER CASE FIGURES INDICATE THE NUMBER OF MICROSPORES; SUBSCRIPT, THE NUMBER OF MICROCYTES IN THE POLLEN GROUP

Species or hybrid	Mean	Types of pollen groups								Total	Anomalous groups
		4 ₀	4 ₁	4 ₂	4 ₃	4 ₄	4 ₅	4 ₆	4 ₇		
<i>Glutinosa</i>	0 01	199	1							200	
<i>Tabacum</i>	0 08	189	6	5						200	
<i>Digluta</i>	0 93	80	77	29	9	3				198	3 ₁ , 3 ₂
F_1 <i>sylvestris-tabacum</i>	0 72	89	76	27	4					196	2 ₀ , 2 ₁ , 2 ₂ , 3 ₁
F_1 <i>tomentosa-tabacum</i>	0 90	82	67	42	8	1				200	
F_1 <i>glutinosa-tabacum</i>	1 12	52	83	45	13	2				195	2 ₁ , 2 ₂ , 2 ₄ , 3 ₁ , 3 ₂
F_1 <i>digluta-glutinosa</i>	2 49	24	42	37	39	34	16	7	1	200	
F_1 <i>digluta-tabacum</i>	2 05	13	57	66	38	22	2	1		199	3 ₁

It may readily be seen, however, that microcyte formation alone cannot possibly account for more than a small fraction of the chromosomal elimination demanded by the results in the foregoing section. Some, but not many, of the microcytes contain more than one micronucleus. If, in order to make allowance for this fact, we arbitrarily raise the average for F_1 *digluta-tabacum* to 2.4 instead of 2.05, then it is easy to show that the average chance of inclusion of each univalent in each microspore nucleus is only reduced from 0.5 to 0.45, somewhat less than one-sixth of the amount of reduction demanded by the foregoing data.

That microcyte formation is not a quantitative measure of chromosome elimination may be demonstrated by direct observation of cells in the quartet stage. In the F_1 *digluta-tabacum* material, in addition to the large microspore nuclei, microspores often contain one or many micronuclei comparable in size to those of microcytes, and they were also observable in various later stages of development of the pollen grain. There seems to be little reason to believe that such eliminated

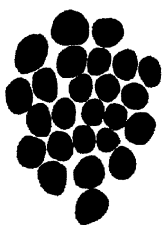


Fig 18

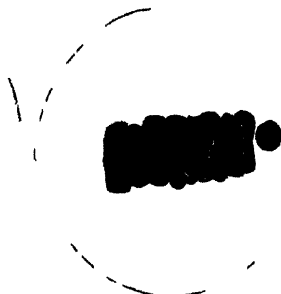


Fig 19

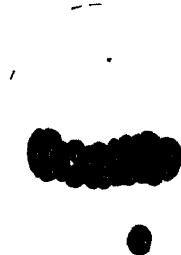


Fig 20



Fig 21

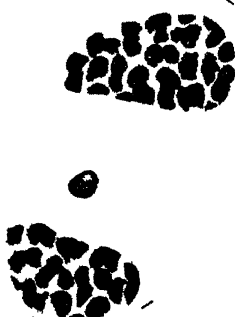


Fig 22



Fig 23



Fig 24

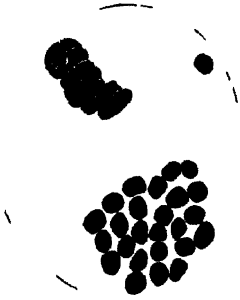


Fig 25



Fig 26



Fig 27 A



Fig 27 B

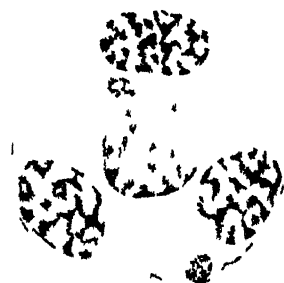


Fig 28

chromosomes ever become reincorporated in the pollen nucleus, so that an enumeration of them may be expected to give an accurate indication of the extent of elimination. It was not feasible, however, to make such an enumeration with confidence in the available material. Confusion may also arise from a certain amount of union of univalents to form larger micronuclei. In order to avoid these difficulties a rather prolonged study was made of meiotic and post meiotic phenomena in plants having only one univalent on the assumption that the behavior of a single univalent might be followed more accurately in such plants than when a large number was present. Some of the pertinent figures are presented herewith.

At I-M, polar view (fig. 18), the univalent is usually readily detectable lying on the periphery of, but rarely in exactly the same plane as, the equatorial plate. In side view it is seen to assume a variety of positions, two extremes of which are shown; one (fig. 19) with the univalent lying exactly in the plane of the equatorial plate, the other (fig. 20) with it lying far removed from the bivalent group. The ensuing anaphase progresses normally as far as the bivalents are concerned; the univalent is often not detectable, in other instances it lags. In the former case it had probably occupied a position like that shown in figure 20, so that it was simply included in one of the groups of separating bivalent partners. Lagging (fig. 21) may be due to the fact that it occupied a position in or nearly in the plane of the equatorial group. In the case of lagging a variety of consequences may follow. In rare cases the chromosome may divide and half may go to each pole; or it may be so tardy in division that neither half reaches the pole. Or it may start to divide, but both halves may pass together to the same pole; or one half may reach one polar group and the other half may remain outside in the plasma.

At interkinesis, then, we may have only two daughter nuclei; or there may be, in addition to these two, one (fig. 22) or two (fig. 23) micronuclei. When there is only one micronucleus it may be difficult to decide with assurance whether it represents an entire, undivided univalent or only half of one. Consequently counts at this stage are

Figs. 18-23. Distribution of the univalent chromosome in a $24_{II} + 1_{I}$ segregant, 26186P16, in *F. digluta-tabacum*. 18, I-M, polar view, $24_{II} + 1_{I}$. 19, I-M, side view, univalent in plane of equator. 20, I-M, side view, univalent towards one pole. 21, I-A, side view, univalent in process of division. 22, early interkinesis, micronucleus in plasma. 23, late interkinesis, two micronuclei in plasma. 24, II-M, dividing univalent in plasma, countable plate exhibits 24 chromosomes. 25, II-M, half of univalent in plasma, countable plate exhibits $24\frac{1}{2}$ chromosomes. 26, II-A, laggard in one figure, other half-univalent in plasma. 27a, b, late II-A, a laggard in both figures. 28, beginning of furrowing, two micronuclei, one of which will apparently be cut off in a microcyte.

probably only indicative; so that no attempt was made to study it exhaustively. However, examination of 38 interkineses in excellent condition disclosed 21 with no micronucleus, 15 with one, and 1 with two.

It would have been interesting to have had enough counts of both plates at II-M to have been able to place the problem of the division of the univalent during I on a statistical basis; but this requires a large amount of satisfactory material, inasmuch as one must depend upon an unusual orientation of the plates. The possible conditions were all observed; viz., $24 + 0 + 25$, $24\frac{1}{2} + 0 + 24\frac{1}{2}$, $24 + 1 + 24$, $24\frac{1}{2} + \frac{1}{2} + 24$, and $24 + \frac{3}{2} + 24$, end terms representing the metaphase plates and the middle term, chromosomes lying in the plasma. The half-chromosomes in II-M plates were readily distinguishable by reason of their paler color. Figures 24 and 25 exhibit the normal type of orientation, which permits a count of only one plate.

In II-A sometimes both groups exhibit a laggard (fig. 27); sometimes only one, in which case there is often a chromosome in the plasma (fig. 26); or often neither group has a laggard, in which case the first division may have been $24 < > 25$ or sometimes $24 + 1 + 24$ or $24 + \frac{3}{2} + 24$, the latter evidenced by the presence of two chromosomes more or less closely associated in the plasma. Laggards in II-A gave no evidence of division, and they were of the same size and staining capacity as chromosomes of the anaphase groups, consequently they were indubitably halves of univalents.

As a result we may have, in the stage preceding furrowing, four microspore nuclei only, or in addition one or two (fig. 28), or rarely more, micronuclei, these latter each representing half of a univalent. Completion of division of the PMC results in production of four microspores, some of which may exhibit micronuclei (fig. 29) or there may be one (fig. 30) or two, rarely more, microcytes in addition to the microspores.

Enumeration of micronuclei is relatively easy and probably most significant at any period from that represented in figure 28, or slightly before, up to the time at which the microspores are released. Counts made on two $24_{II} + 1_I$ plants at about the stage of figure 28 gave the results contained in table 3. For comparison, a count of micronuclei from one of these plants at the stage of figure 30 is also included, together with a count of microcytes in the same cells. Evidently, as stated before, microcyte formation represents only a small proportion of the total elimination. It will also be observed that there is a small

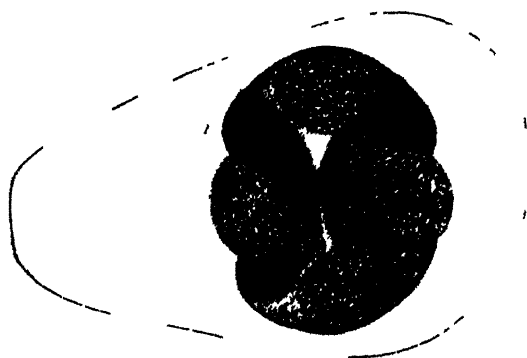


Fig. 29.



Fig. 30.

Figs. 29 and 30. Pollen groups of 26186P16, a $24_{II} + 1_I$, segregant in F_2 *digluta-tabacum*. 29, pollen group exhibiting two micronuclei but no microcyte. 30, pollen group with one microcyte.

TABLE 3

MICRONUCLEUS FREQUENCIES IN TWO $24_{II} + 1_I$ PLANTS. UPPER CASE FIGURES INDICATE THE NUMBER OF MICROSPORE NUCLEI; SUBSCRIPT, THE NUMBER OF MICRONUCLEI, EXCEPT IN THE LAST LINE WHERE THEY INDICATE THE NUMBER OF MICROCYTES

Garden number	4 ₀	4 ₁	4 ₂	4 ₃	4 ₄	4 ₅	Total	q
26186P16, late II	59	70	50	3	2	1	185	0 254
26187P33, late II	63	74	63	7	1		208	0 240
26186P16, pollen groups	56	76	62	3	2	1	200	0 235
26186P16, microcytes	161	35	3	1			200	

proportion of cells with more than two micronuclei. These must be due to simultaneous irregularities in the distribution of bivalents, not to a second division of the univalent. Computations based on these figures, considering cells with more than two microcytes to belong to the 4_2 class, give the proportion of 25-chromosome microspore nuclei shown in the last column of the table under q . The results of these three independent counts are in satisfactory agreement, and their average, 0.243, approaches the value, 0.19, obtained in the preceding section from a study of the distribution of chromosomes in backcross progenies. Since any mistakes in these figures are likely to be due to failure to detect all the micronuclei, the difference between the two values may not be significant. It therefore may be concluded that direct examination of the fate of the univalent bears out the contention that the form of the backcross distribution is mainly due to chromosomal elimination and not to inviability.

The results presented herewith are by no means novel. Almost every investigator who has studied the matter in species hybrids has commented upon the irregularities attending formation of pollen cells. Observations on the production of micronuclei are also abundant, although these small nuclei do not seem to show up so well in iron-haematoxylin slides as in aceto-carmin smears. Rarely, however, has any attempt been made to arrive at a quantitative estimate of the extent of chromosome elimination and its effect upon the gametic series, despite the effect that it must have on breeding behavior.

The most ambitious study of this character is that made by Watkins (1924) on two F_2 plants from a cross of Rivet, a variety of *Triticum turgidum* (14_{II}), with Swedish Iron, a variety of *T. vulgare* (21_{II}), one of which, plant A, was $14_{II} + 3_I$ and the other, plant B, $17_{II} + 4_I$ as to chromosome number. On the basis of counts of losses in the first and second divisions, together with estimates of the chances of recovery during the second division of chromosomes lost during the first, he arrived at the following final expressions for determining the fate of the univalents:

Plant A, $(0.76 + 0.24)^3$

Plant B, $(0.675 + 0.325)^4$

i.e., the chances of a given univalent's inclusion in each microspore nucleus in these cases were 0.24 and 0.325 respectively. While this rather complicated method is no doubt useful for determining the complete history of univalents during meiosis, nevertheless it is not at all clear, even from Watkin's data, that it has any advantage over

direct counts of loss after completion of the meiotic divisions as a method of determining the final chances of a univalent's inclusion in a microspore nucleus. In both of the above plants Watkins presents counts of lost chromosomes in cells of the tetrads, from which the following expressions have been derived:

$$\text{Plant A, } (0.786 + 0.214)^3$$

$$\text{Plant B, } (0.654 + 0.346)^4$$

The value for inclusion in plant A is here somewhat lower, that for plant B somewhat higher, than those given by Watkins, consequently the differences are probably not significant. Watkins favors the former method because of his belief that some of the lost chromosomes may degenerate before the tetrad stage, but the above figures hardly support his contention; and in our own material degeneration would appear to occur at a much later stage, if at all.

The division of the pollen cell would probably be the most satisfactory stage of all for these determinations; but few counts have been made on a statistical basis. Täckholm (1922), however, reports a series of 63 counts of chromosome numbers in pollen cells of two roses of the Canina section, *Rosa tomentella* and *R. Seraphini*, both of the type, $7_{II} + 21_I$, which indicate a strongly pronounced elimination ratio; viz.,

Chromosome numbers	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
Number of cells	9	23	13	7	4	1	1	—	1	—	1	1	—	1	—	1	1

Considering only the first seven classes, the appropriate equation is

$$(0.92 + 0.08)^{21},$$

but the presence of scattering representatives in the higher classes must point to the operation of some other phenomenon.

If we turn to progenies from plants of this type, we also find a marked excess of plants in the lower classes. For this purpose F_2 progenies are not acceptable because it has been thoroughly demonstrated that the effective male gametic series differs strikingly from the female. I know of no instances strictly comparable to the above, in which a plant with univalent chromosomes has been crossed back as a female parent to the corresponding male form without univalents. In the usual studies of species hybridization, the homologues of the bivalents differ genetically, which may introduce an additional disturbing element difficult to evaluate. The nearest approach to the above situation is afforded by crosses of triploid♀ \times diploid♂, such as have been studied extensively in *Datura* and *Oenothera*. The results

of Blakeslee (1924) and his associates on the former and those of Bodeijn (1925) and Dulfer (1926) on the latter all exhibit a marked preponderance of individuals in the lower classes. These authors, however, ascribe the distortion of the distribution to selective elimination of zygotes on account of inviability, a feature which may be more important in their material than in ours. In *Datura* this conclusion is apparently substantiated by the evidence from a statistical study of chromosome numbers in progenies of triploid♀ × diploid♂, which yielded the following results:

Number of chromosomes	24	25	26	Total
Number of plants	215	381	101	697

But in the *Oenothera* material (cf. data in table 4), the value of q is higher than in the *Nicotiana* data and the higher classes are almost entirely unrepresented, which indicates the occurrence of chromosomal elimination.

TABLE 4

CHROMOSOME NUMBERS IN INDIVIDUAL PLANTS OF PROGENIES FROM TRIPLOID ♀ ×
DIPLOID ♂ IN *OENOTHERA*

Authority	Number of chromosomes								Total	q
	14	15	16	17	18	19	20	21		
Dulfer (1926)	3	26	42	20	13	3	4	0	120	0.34
Calc (0.66+0.34)*	6.5	23.6	36.6	81.3	16.1	5.0	0.8	0.1	120	0.34
Bodeijn (1925)	3	35	19	13	3	4	4	0	81	0.30
Bodeijn (1925)	6	45	15	3	4	10	4	2	89	0.30
Total observed	9	80	34	16	7	14	8	2	170	0.30
Calc (0.70+0.30)*	13.8	42.2	54.0	38.6	16.5	4.2	0.7	0.0	170	0.30

Watkins' (1924) contention that chromosomal elimination plays a major rôle in the determination of the gametic series when univalents are present appears to be fully substantiated. However, gametic series may be modified thereafter by selective elimination of certain classes, by competition among pollen classes, and finally the zygotic distribution may suffer further distortion on account of selective inviability. Chromosomal elimination will obviously tend to increase the frequencies of the lower classes; but the other sources of distortion may operate either in the same or in the contrary direction.

F₂ digluta-tabacum

A small F_2 progeny of 25 plants of the *digluta-tabacum* series was also grown and studied individually for chromosome number. Chromosome numbers were determined for 14 plants, with the results recorded in table 5. With one exception, a plant having $25_{II} + 2_I$ chromosomes, all the plants belonged to the $24_{II} + n_I$ series, as in the backcross progeny. There is, however, no reason to suspect that the exceptional plant was incorrectly determined inasmuch as several good figures agreed as to the count. Illustrations of typical chromosome garnitures are represented in figures 31 to 36.

TABLE 5

DISTRIBUTION OF CHROMOSOME NUMBERS IN AN F_2 PROGENY OF THE *digluta-tabacum* SERIES

Garden number	Chromosome numbers, $24_{II} + n_I$									Total
	0_I	1_I	2_I	3_I	4_I	5_I	6_I	7_I	8_I	
26186		3	4	1	2*	2	1	1		14
Calculated	0.4	1.6	2.9	3.4	2.8	1.7	0.8	0.3	0.1	14

*Including one plant which had $25_{II} + 2_I$ chromosomes

While the population was small, it may perhaps be worth while to consider the data from it briefly. The average number of chromosomes in excess of 24_{II} , counting the $25_{II} + 2_I$ plant in class 4, was 3.21, which is 0.96 per plant more than in the backcross progeny. The difference may not be significant; but assuming that it is, the male gametic series must have had an average of $24 + 0.96$ chromosomes each. Unfortunately attempts to secure progeny from *tabacum* ♀ × F_1 *digluta-tabacum* ♂ failed, so that no direct determinations are available. Assuming, however, the correctness of the above value, the observed distribution may be compared with calculated values obtained from the expression,

$$14 (0.81 + 0.19)^{12} (0.92 + 0.08)^{12},$$

which, as shown in the last line in the table, gives an extended range of values similar to the one actually obtained. The standard deviations of the two distributions are also in close agreement, as is shown by the following comparison of constants:

Observed distribution	$M = 3.21 \pm 0.50$	$\sigma = 1.87 \pm 0.35$
Calculated distribution	$M = 3.21 \pm 0.43$	$\sigma = 1.61 \pm 0.30$

The high values of the probable errors, however, make the comparisons of little value.

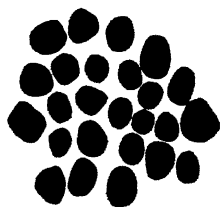


Fig. 31.

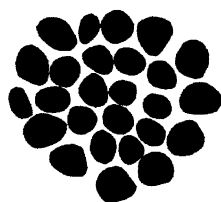


Fig. 32.

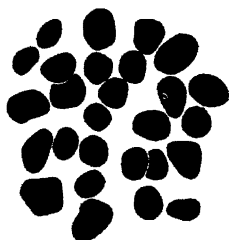


Fig. 33.

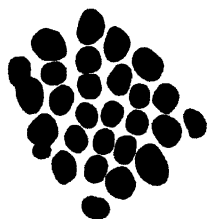


Fig. 34.

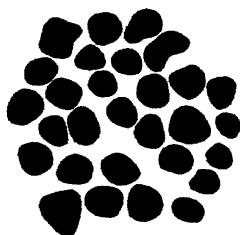


Fig. 35.

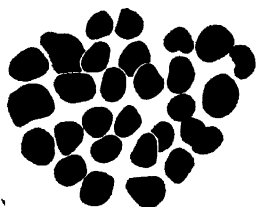


Fig. 36.

Figs. 31-36. *F₂ digluta-tabacum*, I-M plates, polar view, illustrating the $24_{III} + n_e$ series of segregants, n having values from 1 to 6. Note the very small size of one of the univalents in figure 34, which seemed to be characteristic of all figures from this plant.

The fact that only one of the plants had more than twenty-four bivalents is evidently of no significance in this instance, because of the low transmission of extra chromosomes through the male gametes. It does, however, speak in favor of the assumption that there was some transmission through the male gametes. It is probable that such plants will be produced in their expected proportions, and that we are not dealing with a situation such as in the 14×21 chromosome wheat hybrids described by Kihara (1924) and Watkins (1924), in the progenies of which, plants belonging to this category fail to appear.

F_1 *digluta-tabacum* \times *digluta*

A population of 50 plants from F_1 *digluta-tabacum* $\varphi \times digluta$ σ was grown under the garden number, 26184. Results from individual chromosome counts of 26 plants were as follows:

Number of chromosomes	$24_{II}+12_I$	$25_{II}+11_I$	$26_{II}+10_I$	$27_{II}+9_I$	$28_{II}+8_I$	$29_{II}+7_I$	$30_{II}+6_I$
Number of plants	3	3	2	7	4	4	3

The classes of individuals obtained were of the general formula, $m_{II} + n_I$, with m equal to or greater than 24 and $m + n = 36$. Illustrations of typical chromosome garnitures of this series are represented in figures 37 to 42. Assuming that the male gametes all carried 36 chromosomes, the average number of chromosomes in excess of 24 in the female gametes was 3.15 ± 0.36 , with a standard deviation of 1.85 ± 0.26 . Again, in view of the high probable errors of the constants, no extended discussion of the reason for failure to agree with the values of the backcross progeny appears to be justifiable, but it may be possible that the reason lies in irregularities in chromosomal distribution in *digluta*. The results are of interest because they demonstrate that a cross of the type, F_1 *digluta-tabacum* $\varphi \times tabacum$ σ , is the most effective means of doubling up various combinations of the univalent chromosomes contained in F_1 *digluta-tabacum*.

DISCUSSION

From the evidence presented in the previous sections of this paper, the general conclusion has been drawn that *digluta* gives hybrids with its parental species, *glutinosa* and *tabacum*, which exhibit the same general cytological features as certain hybrids between 12_{II} and 24_{II} chromosome species of *Nicotiana*, and that consequently it may be considered a distinct species. It is important to realize, however, that

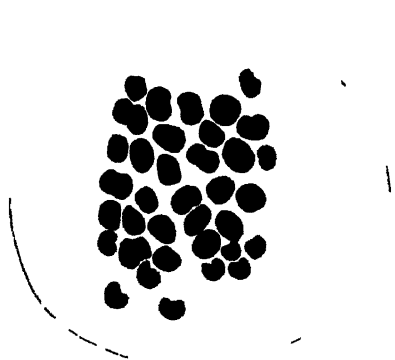


Fig. 37

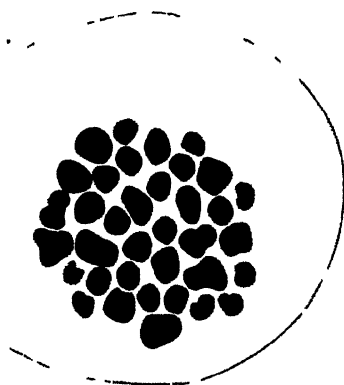


Fig. 38

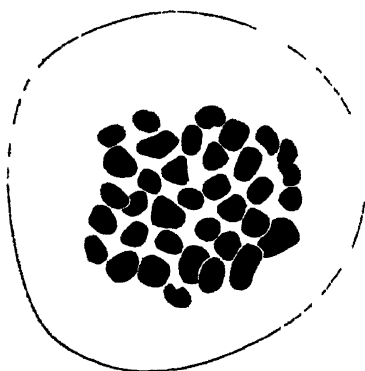


Fig. 39

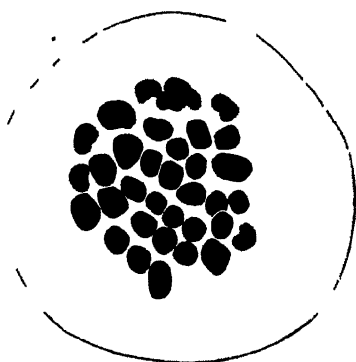


Fig. 40.

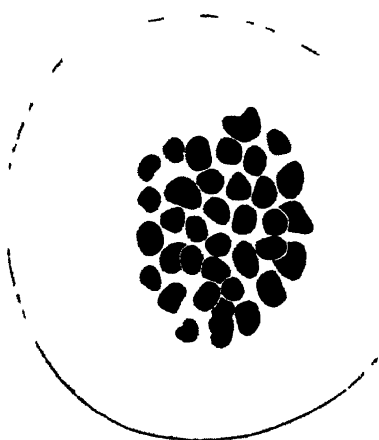


Fig. 41.

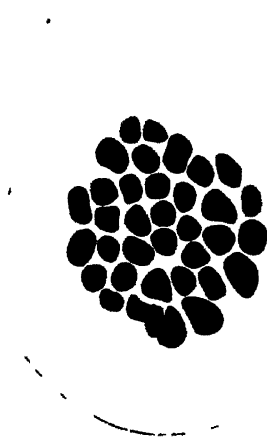


Fig. 42.

Figs. 37-42. *F. digluta-tabacum* ♀ × *digluta* ♂, I-M plates, polar view, illustrating the $m_{II} + n_I$ series of segregants $25_{II} + 11_I$ to $30_{II} + 6_I$.

for actual production of new species in nature according to this scheme, other considerations than the constancy of such types under experimental conditions must be recognized.

The extensive chromosome elimination in F_1 *digluta-tabacum* has the obvious effect that in backcrosses to *tabacum* or in self-fertilization of F_1 there will be a strong tendency to revert to *tabacum*, so strong that one may safely predict elimination of all *glutinosa* chromosomes in the course of two or three generations. Inasmuch as we have observed that *digluta* unprotected exhibits frequent natural crossing with *tabacum*, it follows that, were it to arise in nature, it would stand no chance of survival unless isolated from that species. On the other hand isolation from *glutinosa* is probably not so important; for, although these two species cross readily enough, the sterility of the F_1 hybrid bars it from contributing to the character of the population. Assuming equal survival value, *digluta* might maintain itself in association with *glutinosa*, provided natural crossing of the type, *digluta* ♀ × *glutinosa* ♂ were not distinctly more frequent than reciprocal crossing. Nevertheless, since this appears to be the true situation, *digluta* would tend to be eliminated, but more slowly and in a different way than in association with *tabacum*.

While these objections may with propriety be urged against *digluta* as an effective example of the way in which species with new chromosome numbers may be established in nature, they do not dispose of all similar instances. In particular *Primula kewensis* apparently crosses with such difficulty (cf. Pellew and Durham, 1916) with its parental species, *P. floribunda* and *P. verticillata*, that it is from the beginning effectively isolated from them. The failure of the present instance to exhibit all the phenomena necessary for effective species production does not necessarily demonstrate that the method is generally unavailable for the purpose.

It is obviously an interesting speculative question as to whether any of the existing *Nicotiana* species may have arisen from other known species in a manner analogous to that of *digluta*. In the case of *paniculata* ($12n$) and *rustica* ($24n$) it has been shown (cf. Goodspeed, Clausen and Chipman, 1926) that *rustica* possesses a set of chromosomes cytologically homologous with that of *paniculata*, but differing to some extent in genetic constitution. These latter differences may not, however, be of great moment in view of the notable differences shown among assemblages of *rustica* varieties and further in view of East's demonstration (1921) that segregation products of the *rustica* group largely duplicate existing *rustica* varieties. Accord-

ingly *paniculata* may have contributed one set of chromosomes to *rustica*; but we do not know as yet where the other set came from. The evidence is more complete for *tabacum*, although in some respects less satisfactory. It has been shown that crosses of this species with both *sylvestris* and *tomentosa* give hybrids of the *Drosera* type, $12_n + 12_r$, and that the *sylvestris-tomentosa* hybrid exhibits 24 unpaired chromosomes. Here it is highly probable that one set of *tabacum* chromosomes is homologous with the *sylvestris* set and the

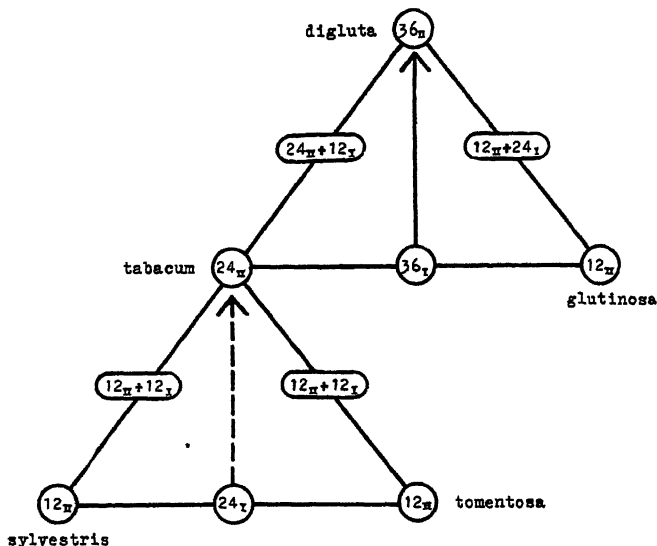


Fig. 43. Graphic representation of the relations of *digluta* to *glutinosa* and *tabacum*, and of *tabacum* to *sylvestris* and *tomentosa*. The arrow with solid shaft in the upper triangle represents the manner in which *digluta* is known to have arisen by doubling of the chromosome number in the F_1 *glutinosa-tabacum* hybrid; the arrow with the broken shaft in the lower triangle represents the manner in which a form cytologically homologous to *tabacum* might arise by doubling of the chromosome number in an F_1 *sylvestris-tomentosa* hybrid.

other with the *tomentosa* set, especially as *tabacum* haploid (Clausen and Mann, 1924) exhibits no chromosome pairing. But it is probable that both *sylvestris* and *tomentosa* chromosomes differ profoundly in a genetic sense from their *tabacum* homologues. Nevertheless, as is well shown in the diagram of figure 43, there is such a close parallelism between the relation of *tabacum* to these two species and that of *digluta* to its parental species as to suggest that *tabacum* may have arisen either from the progenitors or from close allies of *sylvestris* and *tomentosa*. The cytological affinity is still so strong that a form arising from doubling of chromosome number in a *sylvestris-tabacum* hybrid may be expected to give 24_n hybrids with *tabacum*.

SUMMARY

1. Arguments are presented in support of the contention that *N. digluta* (36_{II}) arose from doubling of chromosome number in an F_1 hybrid zygote of *glutinosa* (12_{II}) \times *tabacum* (24_{II}).

2. F_1 *digluta-glutinosa* exhibits 12_{II} + 24_I in meiosis, with univalents distributed for the most part undivided in I and dividing in II.

3. F_1 *digluta-tabacum* exhibits 24_{II} + 12_I chromosomes in meiosis, with univalents distributed as in F_1 *digluta-glutinosa*.

4. Backcross progenies of F_1 *digluta-tabacum*♀ \times *tabacum*♂ conform to the series, 24_{II} + n_I , with values of n determined by the expression, $(0.81 + 0.19)^{12}$, the inequality in the two terms of this expression arising through elimination of univalents during meiosis in F_1 *digluta-tabacum*.

5. Studies of microcyte production in pollen groups of various hybrids indicate extensive chromosome elimination; but they do not give a quantitative measure of the total amount because most of the excluded chromosomes are not included in the microcytes.

6. Studies of chromosome elimination in 24_{II} + 1_I segregants give an average value for q , proportional frequency of inclusion of the univalent chromosome, of 0.243, supporting the contention that chromosome elimination plays a leading rôle in determination of the gametic series in plants having univalent chromosomes.

7. Of fourteen F_2 *digluta-tabacum* plants, thirteen conformed to the series, 24_{II} + n_I , the other was 25_{II} + 2_I. The presence of this plant and the average value of n indicate a low ratio of transmission of univalents in male gametes.

8. A progeny of F_1 *digluta-tabacum*♀ \times *digluta*♂ conformed to the series, $m_{II} + n_I$, m exhibiting values equal to or greater than 24 and $m + n = 36$. Values of m were somewhat above expectation based on the F_1 *digluta-tabacum*♀ \times *tabacum*♂ series, which may be due to irregularities in chromosomal distribution in *digluta*.

9. Although the foregoing data indicate that *digluta* behaves like a distinct species, it probably could not survive under natural conditions unless isolated from the parental species. *Primula kewensis*, on the other hand, is effectively isolated from its parental species because it crosses with them so infrequently.

10. Evidence is presented for the conclusion that *paniculata* (12_{II}) may have been one of the progenitors of *rustica* (24_{II}) and that *tabacum* (24_{II}) may have arisen from hybridization of progenitors or close allies of *sylvestris* (12_{II}) and *tomentosa* (12_{II}).

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CHROMOSOME NUMBER AND MORPHOLOGY IN NICOTIANA

II. DIPLOIDY AND PARTIAL DIPLOIDY IN ROOT TIPS OF TABACUM HAPLOIDS

BY

MABEL LOUISE RUTTLE

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INTRODUCTION

In the course of the *Nicotiana* investigations carried on at the University of California, there developed the need of knowledge of somatic chromosome number and morphology of the various species. The first of a series of studies along this line dealt with *Nicotiana alata* var. *grandiflora* (Ruttle, 1927). Here, although the individual chromosomes could not be identified, as was possible in certain *Crepis* species (Nawaschin, 1927), they could, nevertheless, be arranged into groups on the basis of size and the occurrence of satellites. The present study is concerned with the matter of diploidy in root tips of *tabacum* haploids.

Nicotiana tabacum has been used in many cytological and genetic investigations. Some knowledge of its chromosome morphology is therefore of particular interest. Obviously a haploid rather than a diploid individual should furnish more favorable material for such a study because of the smaller number of chromosomes involved. In our cultures was one haploid plant, found in an F_1 *tabacum* var. *purpurea-sylvestris* population in 1923 and maintained by vegetative propagation. This past summer five additional *tabacum* haploids appeared. Haploidy was determined by IM counts of aceto-carmines smears of PMC and by root-tip counts. A description of these new haploids and the results obtained from a study of their root tips and those of the diploid is recorded below. The frequent occurrence of diploid and partly diploid root tips on haploid plants is illustrated and discussed. Minor studies on anthers and ovules are included.

While haploid Angiosperms have doubtless been occurring both in plant cultures and in nature, they have been recognized as such only within the past six years. In 1922, Blakeslee, Belling, Farnham, and

Bergner reported the occurrence of two haploid plants in their *Datura* cultures. These were found in a highly aberrant population of *Datura*, obtained from the seed of plants exposed to cold. Blakeslee and Belling (1924) and Belling and Blakeslee (1922, 1927) reported fifty such haploid individuals, some of which were obtained from the application of pollen from *Datura ferox* to the stigmas of diploid *Datura stramonium*. The root tips showed the haploid chromosome number 12. In the meiotic division, the chromosomes remained unpaired, were distributed at random in the first division, and divided normally in the second division. The pollen grains so produced degenerated. "Non-reduction" divisions which resulted in the formation of dyads with a normal chromosome number were also not uncommon. "The selfed progeny of the haploids are diploids; arising from non-reduction in the pollen, and doubtless also in the megaspores" (Belling and Blakeslee, 1927, p. 361).

Gaines and Aase (1926) reported a haploid wheat plant in a cross between *Triticum compactum humboldtii* and *Aegilops cylindrica*. This plant was like the female parent but sterile. The meiotic behavior was, in general, similar to that of the *Datura* haploids except that some or all of the chromosomes divided occasionally at the first division, in consequence of which the second division became irregular. In some locules, also, a fusion of the PMC occurred, which resulted in the formation of giant multinucleate "pollen grains." In ovaries and anthers the fusion of two somatic nuclei and subsequent division occurred frequently.

Joergensen (1928) by pollinating *Solanum nigrum* with *S. luteum* obtained one *nigrum-luteum* hybrid, and seven haploid and twenty-eight diploid *S. nigrum* plants. Also, in the progeny from periclinal fruits of the chimera *S. nigrum* var. *gracile* covered by a one-layered epidermis of *S. sisymbriifolium* was one haploid *S. nigrum* var. *gracile*. In the diploid ($2n=72$) behavior was regular. In the haploid ($2n=36$) some variable pairing occurred in consequence of which the reduction division approached the type 12_{II} and 12_I . In the megaspore, trisomes are sometimes formed. Somatic chromosomes from the root tips of both haploid and diploid plants are shown. The diploid cells are considerably larger than the haploid cells.*

From the *Nicotiana* cultures, one *tabacum* var. *purpurea* and one *tabacum* var. *macrophylla* haploid have been described (Clausen and

* Lesley and Frost (Am. Nat., vol. 47, pp. 22-33, 1928) have recently described a *Matthiola* haploid with one additional chromosome fragment.

Mann, 1924). The *purpurea* haploid occurred in an F_1 *purpurea-sylvestris*, numbering 58 plants, and the *macrophylla* haploid in an F_1 *macrophylla-sylvestris*, numbering 50 plants. One of these plants, 23083P57, was made the subject of a detailed study in meiotic behavior (Chipman and Goodspeed, 1927). They found that in the haploid the chromosome initials remain single from the time of the inception of synizesis to the first metaphase, whereas those of the diploid become double between synizesis and second contraction. The chromosomes in the haploid are usually distributed at random at the first metaphase and divide at the second, but division of some or all univalents sometimes occurs at IA, the latter resulting in the formation of dyads. A few "bivalents" appear in some PMC, but since there was no evidence of pairing at diakinesis or earlier, this is probably a mechanical effect.

In *Datura*, *Nicotiana*, and *Triticum* haploids the meiotic behavior therefore corresponds fairly closely. Pairing, if present at all, is probably only the result of a mechanical adhesion and does not represent true pairing. In contradistinction to these, in *Solanum* the pairing is probably true, approaching the type exhibited by many triploids.

This past summer three additional *tabacum* var. *purpurea* and two *tabacum* var. "Cuba" haploid plants appeared in the *Nicotiana* cultures. Two of the *purpurea* haploids occurred in an F_1 *purpurea-sylvestris* population numbering 590 plants. The other *purpurea* haploid occurred in F_1 *purpurea-tomentosa* numbering 26 plants, and the Cuba haploids in an F_1 *Cuba-sylvestris* numbering 320 plants.

In external morphology the three *purpurea* haploids are identical with one another and with 23083P57. The description given for the latter by Clausen and Mann (*loc. cit.*) applies in its entirety to these new haploids except that in them the length of the corolla tube is slightly greater—44 mm. as compared with 40 mm. The diameter of the limb is approximately the same in all cases. Photographs of 27174P5 and of 27157P220 are included (figs. 1 and 3). The former is shown in marked contrast to 27157P436 (fig. 2), a typical *purpurea-sylvestris* hybrid. F_1 *tabacum-sylvestris* is described by Goodspeed and Clausen (1917*a* and *b*) as a replica on an enlarged scale of its particular *tabacum* parent. The haploids are replicas on a reduced scale of their particular *tabacum* parent. This point is illustrated in figure 3, where the haploid 27174P5 is shown beside a normal F_1 *tabacum-tomentosa* hybrid.

The two Cuba haploids bear the same general resemblance to Cuba that the *purpurea* haploids bear to *purpurea*. As compared with Cuba they are much shorter and more slender, with smaller leaves and flowers. The flower measurements in table 1 illustrate this difference in size.

TABLE 1

SPREAD AND LENGTH OF COROLLA IN *tabacum* CUBA HAPLOIDS AND DIPLOIDS;
F₁ *Cuba-sylvestris* AND *sylvestris*

Garden numbers	Classification	Corolla length in mm.	Corolla spread in mm.
27050	<i>sylvestris</i>	85	42
27040	Cuba	45	25
27146	Cuba- <i>sylvestris</i>	55	35
27146P172	Cuba-haploid	36	22
27146P230	Cuba-haploid	36	22

The two *tabacum* Cuba haploids are of some interest. Cuba has been described by Goodspeed (1915) in connection with the parthenocarpic tendency which it exhibits. This tendency is equally manifest in F₁ *Cuba-sylvestris*, which, although sterile, retains and develops its capsules normally, instead of abscissing them early as does F₁ *purpurea-sylvestris*. This retention and development of seed capsules is also marked in the Cuba haploids, in contrast to the *purpurea* haploids in which the capsules are abscised. In this connection it may be noted that in *purpurea* haploid EMC from 27157P220, the chromosomes often all divide at the first metaphase, in which case a diploid egg may be produced. Dyads have frequently been observed in *purpurea* PMC. It is possible, if dyad formation occurs in the Cuba haploids, as in the *purpurea* haploids, that, owing to the retention of the capsules, some selfed seed can be obtained.

METHODS

This considerable group of haploid plants furnished abundant material for chromosome studies in *tabacum*. Root tips obtained from cuttings of four of the haploid plants were killed and fixed in formalin chrom-acetic solution and also in Taylor's modification of Flemming's solution. The former fixative proved in general to be the more satisfactory although in it the chromosomes are slightly more contracted than in the latter. Ovaries and PMC of 23083P57, 27157P220, and 27146P230 were fixed in formalin chrom-acetic. The sections of the root tips, EMC and PMC, were stained almost

exclusively in Haidenhain's iron-haematoxylin. Gentian-violet-iodine proved of value in chromosome studies of PMC and EMC, but was not so satisfactory for root tips. Diploid *tabacum-purpurea* was used throughout for comparative study.

CHROMOSOME MORPHOLOGY

The chromosome number of *tabacum* has been repeatedly recorded as $n = 24$, $2n = 48$. Christow (1925) figured the somatic complex of *tabacum*. Rybin (1927) clearly figured the somatic chromosomes of *N. tabacum* vars. Felix and Dubek, and of *N. rustica*, as well as of the tetraploid and triploid hybrids between them. He found that the chromosome complexes in the two *tabacum* varieties used were similar. The size and shape of the chromosomes varied according to their location in the root tip, being much shorter and less bent in the root cap, as contrasted with the meristematic regions behind. In both the somatic and meiotic metaphases the chromosomes showed size distinctions, and in the somatic metaphases usually formed V's or J's.

Early in the present study the size differences, as noted by Rybin, became perfectly evident, in both diploid and haploid somatic and meiotic complexes. The variation in chromosome length and thickness, depending on the location of the plate in the root tip, noted by him, was also recorded. On the other hand, even in the haploid it proved impossible to arrange the chromosomes in groups on the basis of length and the occurrence of satellites, as was done in *alata* (Ruttle, *loc. cit.*). In the somatic complex, even in the haploid plants, the chromosomes were seldom well separated on the equatorial plate, but were bent and twisted in such a way that only a few long chromosomes could be distinguished with certainty from those of intermediate length. In figure 1*a*, from a root tip of 27174P5, the section was crushed by applying slight pressure under the microscope, so that the 24 chromosomes are exceptionally well separated. In this plate at least 7 long and 2 shorter chromosomes can be distinguished, the remainder being intermediate in length. Some of these intermediate chromosomes are doubtless longer than they appear. Figure 1*b* is from 27157P220, another *purpurea* haploid, and, except that the chromosomes are not so well separated, in consequence of which the long chromosomes and one satellite do not show so clearly, is indistinguishable in chromosome morphology from figure 1*a*. The Cuba haploid complex shown in figure 2*a* is also indistinguishable from the

purpurea complexes. The diploid somatic complex with 48 chromosomes is shown in figure 3a and, like the haploid, is characterized by long bent chromosomes and shorter, less bent ones.

As in *alata*, certain chromosomes of the complex bore small proximal satellites. This morphological character of specific chromosomes of *tabacum* was, apparently, not observed by Rybin. In all the haploid plants examined, at least two such chromosomes were found in certain plates. Consequently, there ought to be at least four



Fig. 1. *Nicotiana tabacum* var. *purpurea* haploid, somatic mitoses, from root tip, showing polar views of two equatorial plates. a, from 27174P5. The chromosomes are well separated and two bear small proximal satellites. b, from 27157P220. The chromosomes are less well separated, one satellite shows clearly, the second (dotted in) shows less clearly. $\times 4500$.

satellited chromosomes in the diploid complex. Up to the present, no more than two have been found with certainty in any one diploid plate, but this can be accounted for by the number and crowding of the chromosomes. Even in the haploid complex, although one satellited chromosome is found commonly, two are found rarely, as is indicated in figures 1a, b, and 2, in only one of which the two satellites are clearly visible. This is, however, no indication that the satellites are not present at all times in all the haploid plants, but simply shows how seldom plates are found in which the chromosomes are sufficiently well separated for the satellites to be distinguishable.

The large number of V- and J-shaped chromosomes may be taken as an indication that the fiber attachment is often median or sub-

median. Transverse clear areas described in *alata* were usually not visible in *tabacum*. In one series of slides, however, they were clear. In this case, moreover, the sections had not been crushed under the microscope to separate the chromosomes.

During the study of chromosome morphology in root tips from haploid cuttings, a large proportion of root tips was found which in whole or in part contained the diploid chromosome number. Thus

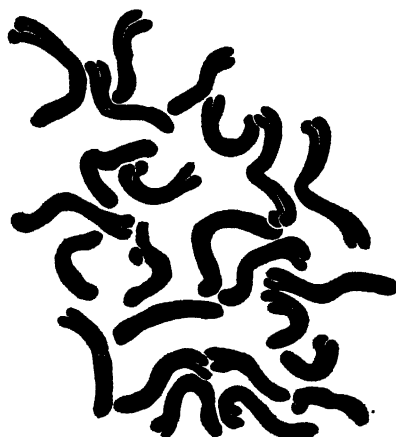


Fig. 2. *Nicotiana tabacum* var. "Cuba" haploid, somatic mitosis, polar view, equatorial plate, showing 24 chromosomes. No satellites are visible in this plate, but are visible in other plates in this root tip. $\times 4500$.

of 82 root tips examined, 52 were haploid, 22 were diploid, and 8 were partly diploid or contained small areas of diploid cells. The plant number, together with the number of root tips examined from each plant and the type of root tips, is given in table 2. The point of origin of diploidy being unknown and perhaps variable, the possi-

TABLE 2
PROPORTION OF DIPLOID AND PARTLY DIPLOID ROOT TIPS OBTAINED FROM
HAPLOID *Nicotiana* CUTTINGS

Plant number	Number of root tips			Total
	Haploid	Diploid	Partly diploid	
23083P57	13	13	5	31
27157P220	15	3	3	21
27174P5				
Cutting 1	13	0	0	13
Cutting 2	1	6	0	7
27146P230	10	0	0	10
Totals	52	22	8	82

bility is not excluded that certain of the roots here classed as diploids may contain haploid tissues in the older regions no longer showing division figures. For the purposes of tabulation, a root tip is counted as diploid when no haploid division figures are found in its growing region.

As shown in table 2, all the root tips of 27146P230 examined were haploid, from 27157P220 only 15 out of 21 root tips were completely haploid, and from 23083P57 less than half the root tips were haploid.



Fig. 3. *Nicotiana tabacum* var. *purpurca*, somatic mitoses, polar views of two equatorial plates, showing 48 chromosomes. *a*, from the root tip of a diploid plant. *b*, from the root tip of a haploid plant. $\times 4500$.

In each case the root tips were from several different cuttings, record of which, unfortunately, was not kept. In 27174P5, however, 13 haploid root tips were from one cutting and 1 haploid and 6 diploid root tips from a second.

In figure 3*b*, in an equatorial plate from a diploid root tip of 23083P57, the 48 chromosomes are shown. The marked similarity between the chromosomes of this figure and of those in figure 3*a* from the diploid plant is evident.

Besides the larger proportion of root tips whose whole meristematic region is diploid, 8 root tips were found which were partly diploid; 3 of these were from cuttings of 27157P220, and 5 from cuttings of 23083P57. The extent of the diploid areas differed in different root

tips. In the root tips from 27157P220, the diploid areas were small, only a few diploid cells occurring in the region of the growing point in the dermatogen and periblem. These cells could be distinguished with certainty from the adjoining haploid cells only at the metaphase. In the meristematic tissue of these roots more distant from the growing point, no diploid cells could be found either in the periblem or in other tissues. It is almost certain that in these cases diploidy arose at the growing point just previous to the time of fixation. The actual origin of diploidy was not observed, nor were cells seen which had unmistakably two nuclei whose fusion might initiate diploidy. Occasionally, however, a nucleus was found whose size and irregular outline (fig. 4*d* at *c*) suggested nuclear fusion.

In the partly diploid root tips from 23083P57 the diploid areas were in general more extensive, even exceeding the haploid areas. The diploid sector was very irregular in cross-section and extended the whole length of the meristematic tissues from the initial cells to the zone of elongation. In at least three of the 23083P57 root tips the diploid cells extended even into the root cap.

In one root tip (photographed fig. 7) the diploid zone was followed from section to section through 102 sections of 8μ in thickness. The photograph (fig. 7) measures 800μ from the first diploid cell noted in the root cap. The root itself, in the region of the growing point, was round, but passing backwards, it gradually assumed the asymmetric shape shown in figure 7. This asymmetry may be correlated with the size of the cells in the diploid and haploid areas. Cell size is not always proportional to chromosome number. There is so much variation in size within a diploid area and within a haploid area that it often confuses the actual size difference between diploid and haploid cells, and the diploid area in figure 7 is therefore outlined only approximately. Diploid cells, however, are on the average somewhat larger than haploid cells.

Drawings of the same magnifications from diploid and haploid and partly diploid root tips of 23083P57 and from diploid *tabacum* var. *purpurea* here included show that the cells and nuclei in figure 4*a* from a haploid root are obviously smaller than those from a diploid root on a haploid plant (fig. 4*b*). These latter are comparable in size to those of 27-014, a diploid *purpurea* plant (fig. 4*c*). In figure 4*d*, from the partly diploid root photographed in figure 7, it is possible to mark the zone between haploid and diploid cells only on the basis of the knowledge gained from the plotting of division figures

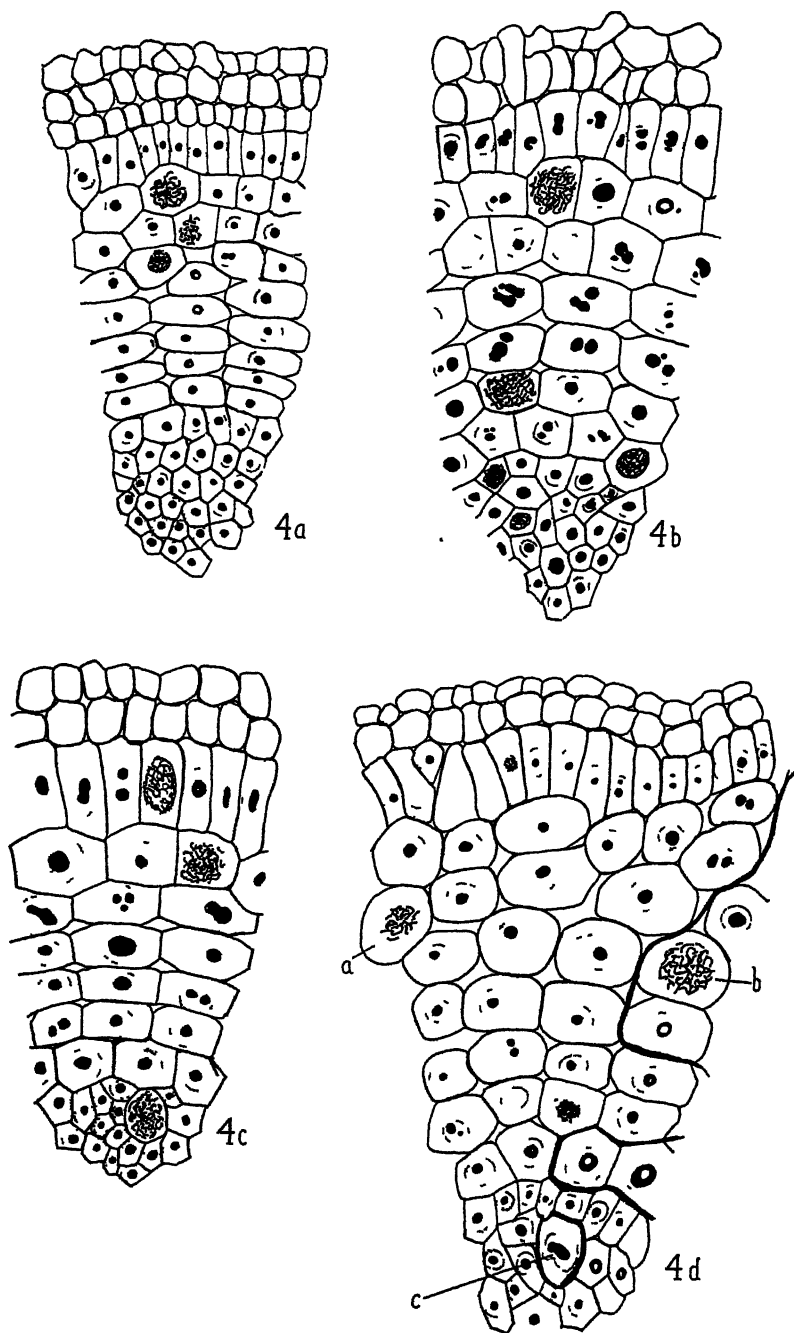


Fig. 4. Sectors from *tabacum* var. *purpurea*, haploid and diploid, to illustrate cell and nuclear size in haploid, diploid, and partly diploid root tips. *a*, a haploid root tip from a cutting of haploid 23083P57. *b*, a diploid root tip from a cutting of haploid 23083P57. *c*, a diploid root tip from diploid *tabacum*. *d*, a partly diploid root tip from a cutting of haploid, 23083P57, showing a haploid cell at *a* and a diploid cell at *b*.

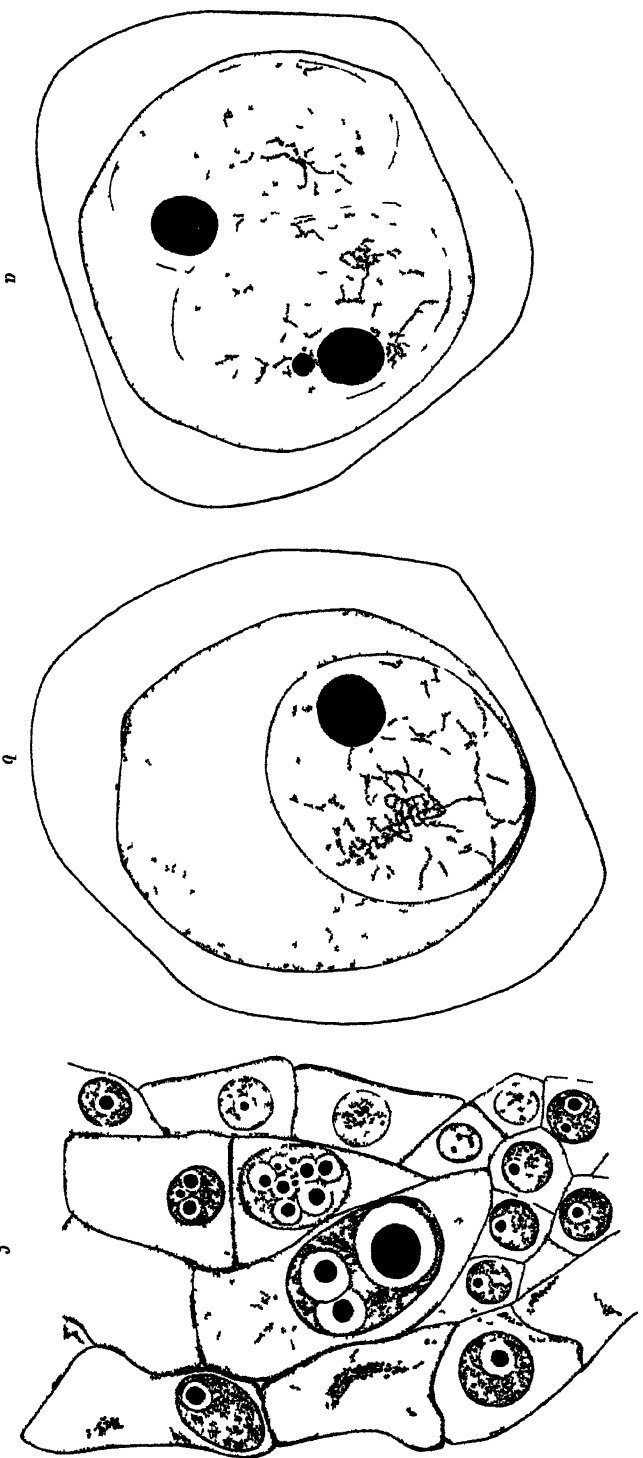


Fig. 5 a and b, two PMC from 28083P⁵⁷ "C₃ tomyxus," is general in this anther site a, a binucleate PMC b, a normal appearing PMC c, a portion of an ovule wall from haploid 27157P²³⁰, showing two exceptionally large nuclei



Fig. 6. a, a haploid *Nicotiana glauca* var. *purpurea*, 27157P220, from an *F*₁ *tabacum-sylvestris* population. b, an *F*₁ *tabacum* var. *purpurea-sylvestris* hybrid, 27157P436. c, an *F*₁ *tabacum* var. *purpurea-tomentosa* hybrid, 27174P4, growing beside a haploid *tabacum* var. *purpurea*, 27174P5.

in previous sections. A haploid cell is shown in division at *a*, and a diploid cell in division at *b*. In the zone between are haploid and diploid cells but only when cells are seen in division in cross-section can the haploid cells be distinguished with certainty from the diploid

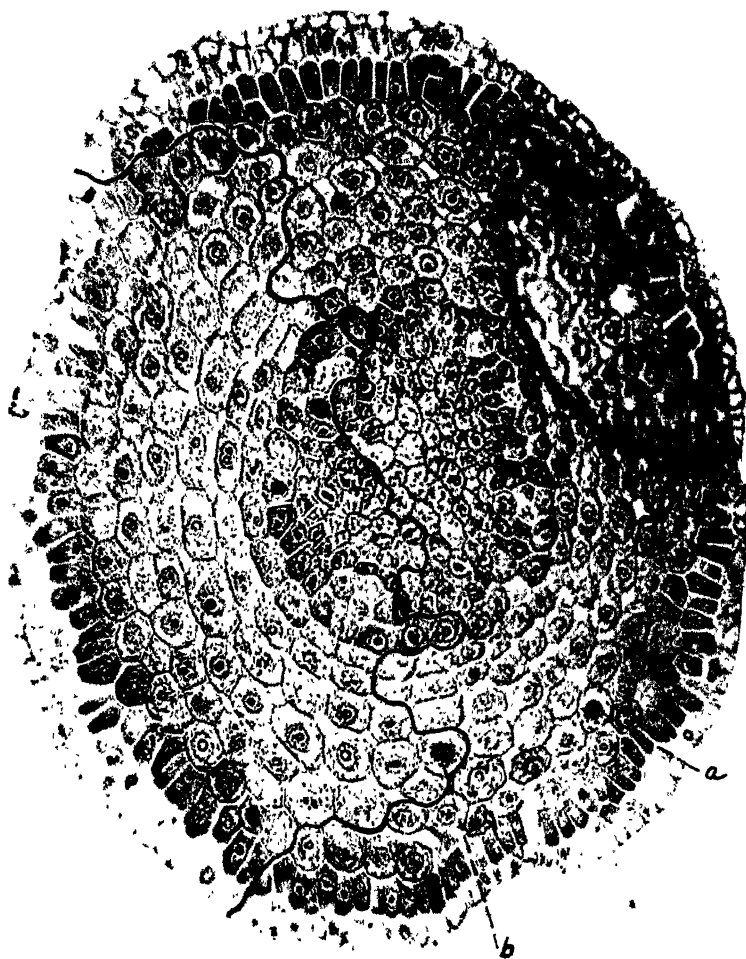


Fig. 7. A *tabacum* var. *purpurea* haploid, 23083P57, root tip in cross-section, showing the diploid sector in outline. At *a*, is a haploid cell, and at *b*, a diploid cell dividing.

cells. For this reason, efforts to trace back diploid lines of cells in longitudinal sections were unsuccessful.

The frequent appearance of diploidy in haploid roots suggested the possibility of tetraploidy in diploid roots. A search was made in diploid *purpurea* root tips, both from cuttings and seedlings, but

no tetraploid cells have so far been found. In general, of course, one might be inclined to feel that diploidy in a basically haploid tissue would be of more frequent occurrence than tetraploidy in a diploid tissue, there being perhaps a tendency to return to, or recover the normal diploid condition.

Although the large proportion of diploid root tips suggested the possibility of diploidy in other parts of these haploid cuttings, no diploid branches or flowers have been found so far. The search was made in archesporial tissue, PMC, and ovules of two haploid plants for diploid as well as binucleate cells. In the archesporial tissue none was found but in certain pollen sacs in which cytomyxis and degeneration were prevalent, occasional binucleate PMC occurred (fig. 5a). These binucleate PMC, like those described by Håkansson (1926) in *Verbascum* and *Celsia*, resulted from the extrusion of the entire nucleus of one PMC into the cytoplasm of an adjoining PMC, rather than of a part of the nucleus as is more common. Extrusions of chromatin were found in aceto-carmine smears as well as in imbedded material. No binucleate PMC were found in anther sacs in which cytomyxis was not occurring. Adjoining the binucleate PMC (fig. 5a) on one side was a PMC (fig. 5b) of normal appearance and on the other side an enucleate PMC. Although these PMC (figs. 5a, b) appear healthy they will doubtless degenerate as the majority in this sac were doing. Fusion of nuclei or parts of nuclei could not be demonstrated but should it occur the accompanying degeneration would prevent establishment of diploidy in this fashion.

In a further attempt to discover diploidy in the meristematic regions of haploid plants, young ovules of *Nicotiana* haploids were examined. The chromosomes, however, were too crowded for any counts to be made, so that although certain nuclei appeared to have more than the normal chromosome number, this could not be proved. An exceptional case was the two large nuclei in the tissue of the ovule wall (fig. 5c). The largest of these nuclei was at least four times as large as that of the nuclei in the surrounding cells and the cell containing it was correspondingly large. The nucleus contained three large nucleoli, the biggest of which was equal in size to the whole of an ordinary haploid nucleus. These two exceptionally large nuclei may have resulted from a nuclear division and subsequent fusion, or merely from disturbed nutrition.

DISCUSSION

The factors operative in the production of haploid plants are unknown. *Datura* haploids were obtained from the seed of plants exposed to cold and also by pollinating *D. stramonium* with pollen from *D. ferox* (Belling and Blakeslee, 1927). All other haploids recorded were obtained as a result of interspecific pollination. The species may cross readily as in *Nicotiana tabacum-sylvestris*, *Triticum-Aegilops*, or with difficulty as in *Solanum nigrum-luteum*. It is probable that in both cases the unfertilized egg is in some way stimulated to develop.

The occurrence of tetraploidy in diploid root tips is sporadic and usually very limited, often being observed in only one or two root tips of many examined. The literature of this subject has recently been reviewed by Langlet (1927) and Hollingshead (1928). Langlet described and figured two tetraploid root tips, one from *Thalictrum aquilegifolium* and one from *T. rariflorum*. Hollingshead reported tetraploidy in the roots of two different *Crepis* plants. "One a *Crepis biennis* \times *C. setosa* hybrid derivative, had one root tip partially tetraploid. The other, a plant of *C. Bureniana*, had two roots wholly tetraploid." On the other hand, Lesley (1925) found, in studying root tips from tomato plants, that from two cuttings and one seedling, many root tips were tetraploid, either wholly or in part, while others were entirely diploid. The only case aside from the present work dealing with diploidy in the somatic tissues of haploid Angiosperms is that in *Triticum* ovules reported by Gaines and Aase (*loc. cit.*).

A comparison of sectorial tetraploidy in root tips studied by Langlet and Lesley and sectorial diploidy in *tabacum* haploid showed much in common. In cross-section, the sectors were irregular in outline. The portion containing the larger chromosome number had, on the average, larger cells than the remainder of the root tip. The area with the doubled chromosome number may extend from 600 μ to 800 μ back from the tip and even forward into the root cap, or, as occurred in three cases of *Nicotiana*, may be present in only a few sections near the growing point. This variability in size and extent indicates that the time, in the history of the root tip, when the chromosome number doubles, varies. The extent involved in the initial change can be judged only by inference. It, too, probably varies

judging by the fact that in some cases the whole root and in other cases only a small sector is involved.

Again, the factors operative in producing polyploidy in diploid plants and diploidy in haploid plants are unknown. Langlet suggested that cuttings are more likely than seedlings to give rise to roots with a tetraploid chromosome number and referred to the numerous tetraploid roots found by Lesley on the two tomato cuttings. But Lesley also found a seedling tomato with many tetraploid roots and considered that cuttings were probably no more apt to have tetraploid roots than seedlings. On the other hand, Joergensen, working on *Solanum*, stated that “. . . tetraploid (and triploid) shoots will appear when decapitated plants are simply allowed to regenerate. . . .”

Lesley stated that the tomatoes studied were slightly infected with mosaic and suggested that the virus may have had some connection with the origin of tetraploidy. Several of the haploid and diploid *tabacum* plants used in the present study were somewhat infected with mosaic. So far as noted, the disease did not affect chromosome behavior in diploid root tips, PMC, or ovules. It seems unlikely therefore that the shifting from haploidy to diploidy in the haploid roots should have been induced by disease, although this possibility should be kept in mind. It is interesting that the cases cited, providing the most abundant material of doubling of the chromosome number, should belong to the *Solanaceae*, and, with the exception of Lesley's seedling tomato, were from cuttings or decapitated plants.

Several explanations as to the mode of origin of tetraploidy in diploid roots have been suggested, the most usual being nuclear fusion following nuclear division without cell wall formation. Langlet ascribed the origin of the tetraploid sectors in *Thalictrum*, and Gaines and Aase the diploid cells in haploid *Triticum* ovules, to this process. The mode of origin of diploidy in the root tips of *Nicotiana* haploids is unknown, no direct evidence having been obtained.

Acknowledgments are made to Professor T. H. Goodspeed for kindly criticism and advice.

SUMMARY

1. Three additional haploid *Nicotiana tabacum* var. *purpurea* and two haploid *tabacum* var. *Cuba* plants appeared this past summer in the *Nicotiana* cultures. These are described and figured.

2. These haploid *tabacum* plants were used for the study of somatic chromosome morphology. Briefer comparative studies were made of diploid *purpurea*. The somatic chromosome complex of haploid *purpurea* and of *Cuba* are indistinguishable. Each consisted of 24 chromosomes showing size differences. At least two of the chromosomes bore small proximal satellites.

3. The diploid *purpurea* somatic complex consisted of 48 chromosomes, showing size differences, certain of which bore small proximal satellites. In no case were four satellites seen in any one plate. The diploid complex was similar, whether from the root of a diploid plant or from a diploid cell in the root of a haploid plant.

4. Of 82 root tips examined from the haploid cuttings, 52 were haploid, 22 were diploid, and 8 contained both haploid and diploid cells. In the latter, the extent of the diploid area varied from a small isolated group of cells in the neighborhood of the growing point to a large sector, extending forward into the root cap and back through the whole length of the meristematic regions. In cross-section, such a diploid zone is very irregular in outline.

5. True diploidy in haploid plants seemed to be limited to the root tips. At least it was not found in archesporial tissue, PMC, or ovules. Owing to "nuclear extrusion," binucleate PMC sometimes occurred, but degenerated before further development could take place. Two exceptionally large nuclei in the tissue of one ovule wall may have resulted from nuclear division and subsequent fusion, or merely from disturbed nutrition.

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CYTOLOGICAL AND MORPHOLOGICAL
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I. CHROMOSOME NUMBER AND MORPHOLOGY
IN SEVEN SPECIES

BY
IRA JUDSON CONDIT

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INTRODUCTION

The species of the genus *Ficus*, reported to number over six hundred, are found in tropical and subtropical regions. Few genera of plants show such an exceeding diversity in habit of growth, size, form and shape of leaf, flower characters, and fruit production as do the members of this genus. Botanically they can be classified into groups depending upon the location of the fruit buds, whether fascicled or axillary, and upon the arrangement of the staminate and pistillate flowers. While the group has been studied by several taxonomists and by a few specialists in morphology, cytological studies have been almost entirely lacking.

There is a voluminous literature on *Ficus carica*, its dioecism and the interesting relationship existing between the edible fig and the caprifig, and extensive bibliographies are given by Eisen (1901), Grandi (1920), and others.

Du Sablon (1908) studied the structure and development of the albumen in the ovary of the caprifig. He pointed out that in case a blastophaga egg has been deposited in the female flowers the albumen develops parthenogenetically, its rôle being to nourish the larva. However, he did not recognize clearly the exact structure of the nucellus or embryo sac. In certain preparations of young albumen he noticed mitotic figures but could not distinguish the individual chromosomes.

The present paper deals with the results of a study of chromosome numbers and chromosome morphology in seven species of the genus. Acknowledgment is made to Professor T. H. Goodspeed for suggestions and criticisms during the work.

MATERIALS AND METHODS

The species available were as follows: *Ficus carica* Linn., *F. pseudo-carica* Miq., *F. palmata* Forsk., *F. glomerata* Roxbg., *F. erecta* Thunb., *F. rubiginosa* Desf., *F. elastica* Roxb. Brief consideration may be given to the morphological characteristics and relationships of these species. The first three are apparently closely related, the trees being easily budded or grafted one upon the other. In fact Trabut (1922) concluded, after studying the geographical distribution of the so-called indigenous specimens of *F. carica*, that it is a hybrid between two or three closely related species such as *F. palmata*, *F. virgata*, *F. persica*, and *F. pseudo-carica*. They are all deciduous, at least in California, and are all inhabited by the same species of *Blastophaga*. This insect inhabits the gall flowers of the caprifig, sometimes designated as male fig, and in the adult form carries the pollen from the staminate flowers of the caprifig to the pistillate flowers of the common fig on a separate tree. The caprifigs and the common edible figs of *Ficus carica* are so different in floral characters that some taxonomists considered them different species. Muller (1882), however, agreed with Linnaeus that the male and female forms belong to one and the same species and the results of Solms-Laubach's (1885) study of certain species in Java confirmed this opinion.

Ficus carica is the species which is most widely distributed and of greatest economic importance. *F. pseudo-carica* is a native of Abyssinia and is of interest to California fig growers because of the production of pollen in the fruit of all three crops. However, in California it has never been grown commercially to any extent as a source of pollen except in the Imperial Valley. Recent experience with *F. palmata* seems to indicate that it is a more vigorous grower, a more prolific bearer, and more productive of pollen in all three crops than is *F. pseudo-carica*. The strictly pistillate forms of these two edible, fruit-producing species are rarely found in cultivation in this State.

Ficus glomerata, or cluster fig, is occasionally seen in southern California. The tree, which is deciduous for a short time at the end of winter, bears small dry fruits. *F. erecta* is a native of the Orient whence seeds have recently been introduced into California by the writer. The plants are said to be extraordinarily variable in habit of growth and in foliage. *F. elastica* and *F. rubiginosa* are plants which have thick leathery leaves, with abundant latex in all parts. The latter

species is regarded, by at least one taxonomist, as a variety of the former.

Root tips of *Ficus carica* are very easily secured. Cuttings placed in damp moss or sand root profusely in the course of a few weeks. Potted plants of all the species studied have been available in the greenhouse and root tips were readily procurable from most of them. Seedlings of *F. erecta* in flats were growing vigorously and furnished especially good material for active cell division.

Staminate flowers of *Ficus carica* are abundant in the profichi or spring crop but are scarce or entirely lacking in the mammoni and mamme crops. A series in the development of the anthers was obtained at Fresno on the following dates: April 11, April 18, April 23, April 29, May 5, May 11, and May 18.

Fresh anthers were placed in Carnoy's solution for twenty-four hours, then imbedded in paraffin following the usual alcohol-xytol schedule. Sections were cut 4μ or 5μ in thickness and stained in Haidenhain's iron alum haematoxylin. Evidently meiosis proceeded actively during the periods between May 5 to May 11 and May 11 to May 18. Anthers which were entirely somatic on the earlier dates, showed tetrads and even mature pollen grains in flowers collected on the later dates. Pollen mother-cell material was not found in the regular series collected from three or four varieties of caprifigs but was found in a miscellaneous lot of anthers collected on May 18. Pollen mother cells in division stages were also obtained on the same date from caprifigs of *F. palmata*. Carnoy's solution brought about some plasmolysis of cells both in root tip and anther tissue.

Root tips used in this study were mostly fixed in Karpechenko's solution made up in two parts as follows and mixed in equal proportions as used: first, 40 cc. formalin, 10 cc. water; second, 90 cc. water, 1 gr. chromic acid, and 1 cc. acetic acid.

The root tips, imbedded in paraffin, were sectioned and stained just like the anther material, except that it was necessary to cut some as thin as 3μ . Difficulty was encountered in clearing and bleaching the cytoplasm, which generally remained dense or cloudy even with sections left in commercial peroxide for a period of several hours. Destaining required careful attention. It was found most satisfactory to destain to a certain point, wash for a few minutes, mount in glycerine or nujol with a coverglass, and examine with an oil immersion lens to locate mitotic figures and observe their condition as to detail. In the completed slides it was necessary to explore the field for satisfactory mitotic figures with an oil immersion lens.

CHROMOSOME NUMBER AND MORPHOLOGY

FICUS CARICA Linn.

Root tips of several different varieties of the common fig were available. These included common figs such as the Kadota, figs of the Smyrna type such as the Calimyrna and Biskra, a few caprifigs, and some aberrant forms such as the Cordelia, a type of edible caprifig, and the Hamma with flowers intermediate between the long-styled flowers of the common fig and the short-styled flowers of the caprifig.

Mitotic figures were found in the periblem and in the plerome. The chromosomes were generally more clearly and sharply defined in the former than in the latter.

The prevailing number of chromosomes in the somatic cells of *Ficus carica* was determined to be 26. Of forty-six cell plates of common figs, thirty-three showed very definitely 26 chromosomes; nine showed 25, two showed 24, and two a possible set of 28 chromosomes each (pl. 7, fig. 2). Of seventeen cell plates of the Biskra, a variety of the Smyrna type, twelve definitely showed 26 chromosomes, three 25, one 24, and one possibly 28 chromosomes (pl. 7, fig. 3). Of eighty-one cell plates of caprifigs, forty-nine definitely showed 26 chromosomes, seventeen 25, eleven 24, and four possibly 27 or 28 chromosomes (pl. 7, fig. 1).

The individual chromosomes are extremely minute. The longest are not much over 2μ long while the smallest are considerably less than 1μ in length. The length of the larger individuals is several times greater than the diameter. Some are rod-like, others are slightly bent, while a few are decidedly falcate. The number of falcate chromosomes in any cell plate was not large, varying from two to ten or twelve. The small size of the chromosomes and the difficulty in getting them sharply defined made detailed morphological studies difficult. However, in various plates one or more elongated chromosomes, each with a slight S curve, as illustrated in figures 2, 4, 5, plate 7, were distinguishable. Besides the falcate individuals, there were occasional ones with a hooked or curved end as shown in figure 5 for chromosomes of *F. palmata*. It is doubtful whether these characters are sufficiently distinct or recognizable to be of any importance in separating common figs from caprifigs in the seedling or later stages.

FICUS PALMATA Forsk.

The somatic chromosomes of *F. palmata* appear to be the same in number and in gross morphology as those of *F. carica*. The preparation of root-tip material was not so satisfactory as in the latter species and only a small number were studied. Of seven cell plates examined four showed definitely 26 chromosomes and three showed 25. A very few are falcate, some are elongated, but the majority are about three or four times as long as broad. In some plates a few chromosomes were found, each somewhat resembling a fishhook in shape (pl. 7, fig. 5).

FICUS PALMATA \times CARICA

Root tips of a hybrid between *F. palmata* and *F. carica* were available for study. These were from a potted plant of S.P.I. No. 45235 of the United States Department of Agriculture, a seedling grown from seed furnished by Dr. L. Trabut, Algiers. The somatic chromosomes of the hybrid are comparable in every respect to those of the parents.

FICUS PSEUDO-CARICA Miq.

The somatic chromosome number of *F. pseudo-carica* is undoubtedly the same as that of the two closely related species just discussed, viz., *F. carica* and *F. palmata*. Of eighteen cell plates studied, fifteen showed definitely 26 chromosomes. Morphologically the chromosomes also appear to be similar. Some of the individuals of medium length as well as some of the longest are falcate in shape (pl. 7, fig. 4).

FICUS ELASTICA Roxbg.

This species, the common rubber tree, also appears to have 26 somatic chromosomes. The root-tip material did not clear up or stain so sharply as that of the related species next considered. However, seventeen cell plates were studied. Eight showed 26 chromosomes fairly distinctly, five the same number indistinctly, and the rest showed from 23 to 25 chromosomes each. In length, shape, and general appearance they appeared to be similar to those of the three preceding species (pl. 7, fig. 7).

FICUS RUBIGINOSA Desf.

While the prepared slides of root-tip material of *F. elastica* were rather poor, those of *F. rubiginosa* were exceptionally clear. The number of somatic chromosomes is without doubt 26. Of twenty-six cell plates counted, eighteen clearly showed 26 chromosomes and the remainder one or two less. No definite number of falcate individuals could be counted; some cell plates seemed to have three, some four, and some five sickle-shaped chromosomes while others showed a smaller number (pl. 7, fig. 8).

FICUS ERECTA Thunb.

The somatic chromosome number of *F. erecta* is evidently the same as that of the other species just discussed, viz., 26. Six plates out of twenty studied showed this number clearly while the remainder were indeterminable as to number. Root tips were secured from seedlings growing vigorously in shallow flats and the cells seemed to be unusually active. Figures of prophases were numerous and early metaphase plates were more easily located than in material of most of the other species. However, the making of exact counts was difficult as very few figures showed the chromosomes separate and distinct from one another. Morphologically they appeared to be similar to those of the foregoing species (pl. 7, fig. 9).

FICUS GLOMERATA Roxbg.

The number of somatic chromosomes of *F. glomerata* has not been definitely determined. While in all the preceding species it was possible to find the full complement of chromosomes in one plane or by slight focussing up and down, such was not the case with this species. The first counts made of various cell plates indicated a smaller number of chromosomes than in the six species just enumerated. Of twenty-five cell plates studied, from 18 to 20 chromosomes could be definitely counted. A more careful examination of the same figures showed some which undoubtedly had 24 chromosomes, and possibly 26. Without more abundant somatic material and also PMC preparations, only one conclusion can be made: that the somatic chromosomes of *F. glomerata* are more than 20 and probably number 24.

The morphological characters of the chromosomes of this *Ficus* are strikingly different from those of the other six species. The individual chromosomes are globular or oblong (pl. 7, fig. 10), as contrasted to the elongated, rod-like, or falcate chromosomes of the others. The average chromosome diameter of this species does not exceed 1μ . Some of the oblong individuals are over 1μ in length. On account of their minute size and their positions it was often difficult or entirely impossible to determine whether an object was a single oblong chromosome, a chromosome of a dumbbell shape, or two chromosomes lying side by side. The individual chromosomes of this species strongly resemble the bivalent chromosomes of *F. carica* and *F. palmata*.

As previously explained, pollen mother cells were obtained from caprifigs of *Ficus carica* and *F. palmata* only. Staminate flowers are common only in the profichi or spring crop of *F. carica*. In *F. palmata* and *F. pseudo-carica*, staminate flowers are numerous in the mamme or over-wintering crop maturing at Fresno the first of April, in the profichi figs maturing in June, and in the mammoni figs maturing during August, September, and October. Since in most cases each species of *Ficus* requires a distinct hymenopteron to bring about pollination and the resultant setting of fruit with developed anthers, it will probably be possible to get PMC material of only a very few species under California conditions.

In *Ficus carica* and *F. palmata* the haploid number of chromosomes was found to be 13. No perceptible differences in bivalent chromosome morphology between those of the two species could be found. The globular chromosomes are either well separated or grouped in twos or threes (pl. 7, figs. 11, 13).

TAXONOMIC CONSIDERATIONS

The significance which cytological studies of this nature may have in connection with the taxonomy of the species considered has been discussed by various writers within recent years (cf. Clausen, 1927, Heilborn, 1924, Babcock and Lesley, 1926, and Mann, 1925).

It is not within the province of this paper to discuss in any detail the taxonomy of the genus *Ficus*. The species discussed belong to the following groups or sections of King's monograph: *F. carica*, *F. pseudo-carica*, *F. palmata*, and *F. erecta* to the section *Eusyce*;

F. elastica and *F. rubiginosa* to the section *Urostigma*; and *F. glomerata* to the section *Neomorphe*. The chromosome morphology of the four species of *Eusyce* and of the two species of *Urostigma* appears to be very similar, while that of the one species in the section *Neomorphe* is very distinct. Taxonomically the species in sections *Eusyce* and *Neomorphe* have only minor differences. In *Eusyce* the receptacles are mostly axillary while in *Neomorphe* they are mostly in fascicles from stems and branches. King regarded the section *Eusyce* as the most artificial group of the genus and the one least satisfactory. The fact that the chromosome morphology of the four species of this section is similar, can be considered somewhat significant, although a much larger number of species should be studied before attempting to draw any conclusions as to the importance of cytological data in regard to their taxonomic relationships. It is also significant that the chromosomes of one species of the section *Neomorphe* differ so markedly from those of the six species belonging to other sections. *F. glomerata*, included in *Neomorphe*, has monoecious receptacles, as do the species of the section *Urostigma*, to which *F. elastica* and *F. rubiginosa* belong. Studies of other species in the same groups or of species belonging to the sections *Synoecia*, *Sycidium*, or *Covellia* may establish numerical or morphological differences in chromosomes which have not been revealed in the species so far studied.

SUMMARY

1. A review of the available literature fails to show any account of cytological studies of species of *Ficus* in which statements as to chromosome numbers or chromosome morphology are included.

2. The diploid chromosome number for seven species of *Ficus* has been determined as follows: *F. carica*, 26; *F. palmata*, 26; *F. pseudo-carica*, 26; *F. elastica*, 26; *F. rubiginosa*, 26; *F. erecta*, 26; *F. glomerata*, probably 24.

3. The haploid chromosomes of *Ficus carica* and *F. palmata* were studied and found to be 13 in number, all more or less similar in morphological character.

4. The morphological characters of the chromosomes of the first six species listed are similar, the individuals being rod-shaped, falcate, some slightly hooked, or a few doubly curved. The morphological characters of the individual chromosomes do not seem to be sufficiently distinct to enable one to separate seedlings of *Ficus carica* into groups of caprifigs, common figs, or Smyrna figs.

5. The chromosome morphology of *Ficus glomerata*, which belongs to a different section of the genus than any of the other species, is quite distinct, the individual chromosomes being globular or oblong rather than elongated.

6. Four of the species studied, viz., *Ficus carica*, *F. palmata*, *F. pseudo-carica*, and *F. erecta*, belong to the section of the genus, *Eusyce*, according to King's monograph. Two, *F. elastica* and *F. rubiginosa*, belong to the section *Urostigma*; and one, *F. glomerata*, to the section *Neomorphe*. Further cytological studies of species in the same sections or in other sections of the genus *Ficus* may reveal chromosome relations which are of taxonomic importance.

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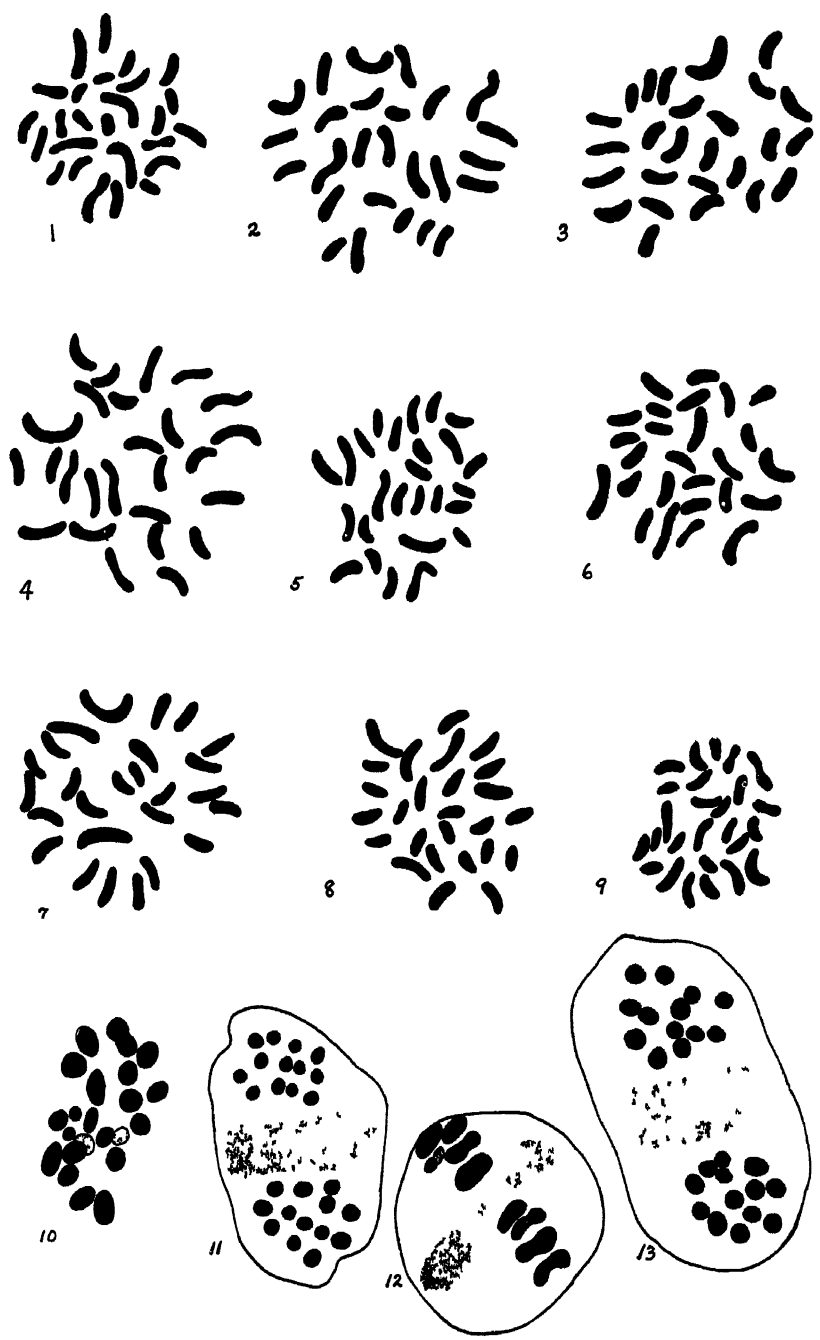
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EXPLANATION OF PLATE 7

Camera lucida drawings of chromosomes of *Ficus* species magnified 4000 diameters, with a 2 mm. oil objective and a 20x compensating ocular.

Figs. 1 to 10, somatic metaphases, polar views as follows: 1, *Ficus carica*, caprifig; 2, *F. carica*, common fig; 3, *F. carica*, Smyrna type fig; 4, *F. pseudo-carica*; 5, *F. palmata*; 6, *F. palmata* × *carica*; 7, *F. elastica*; 8, *F. rubiginosa*; 9, *F. erecta*; 10, *F. glomerata*; 11, PMC heterotypic metaphase, polar view, *F. carica*; 12, side view, same; 13, PMC metaphase, polar view, *F. palmata*.



INTERSPECIFIC HYBRIDIZATION IN NICOTIANA
VIII. THE SYLVESTRIS-TOMENTOSA-TABACUM HYBRID
TRIANGLE AND ITS BEARING ON THE
ORIGIN OF TABACUM

BY

T. H. GOODSPEED AND R. E. CLAUSEN

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In studies of interspecific hybridization in *Nicotiana* we have endeavored to determine whether any consistent cytological behavior may be demonstrated in groups of hybrids. The simplest condition for such a demonstration should be provided by a study of the three possible hybrids which may be obtained between three given species. Thus with the species, *sylvestris*, *tomentosa*, and *tabacum*, the three possible hybrids, F_1 *sylvestris-tabacum*, F_1 *tomentosa-tabacum*, and F_1 *sylvestris-tomentosa*, have been obtained and studied. We call such a series a hybrid triangle. Despite the fact that some forty interspecific hybrids have been obtained in *Nicotiana*, very few complete hybrid triangles are represented among them. The one dealt with in this paper, however, is of special interest because of the significance it may have for the origin of *tabacum*.

F_1 MORPHOLOGY

F_1 *sylvestris-tabacum* is characterized by a remarkable resemblance to its *tabacum* parent, as is shown by the fact that a wide range of highly distinct *tabacum* varieties give F_1 hybrids with *sylvestris* which exhibit with great fidelity the characteristic features of their particular *tabacum* parents on an enlarged scale. Despite the recent contentions of Brieger (1928) as to the characteristic features of these hybrids, we believe that the evidence set forth in our own account (Goodspeed and Clausen, 1917) is adequate and convincing; moreover, our subsequent observations are in harmony with it.

F_1 *tomentosa-tabacum*, on the other hand, clearly represents a synthesis of the characteristic features of the parental species, as may be appreciated by examination of plate 8, figure 2, where it is shown beside a *tabacum* haplont. Its habit approaches that of *tomentosa*, although it apparently never grows so large nor so tall, and it is more

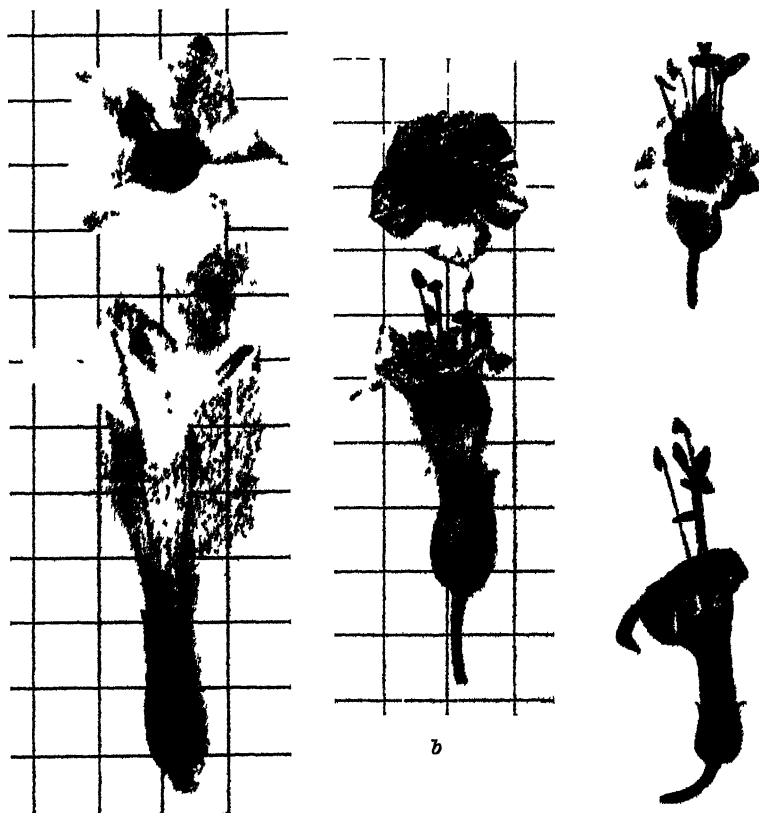


Fig. 1. Flowers of (a) F_1 *sylvestris-tomentosa*, (b) F_1 *tomentosa-tabacum*, and (c) *N. tomentosa*. $\times \frac{9}{10}$.

or less strictly annual as contrasted with the perennial character of that species. The flowers are somewhat larger than those of *tomentosa*, but they are very much like them in form as is manifested by their bilabiate tendency, reflexed corolla lobes, and exserted style and stamens (fig. 1b). The color is more pronounced than that of *tomentosa*, but it is a rusty shade of red, not the clear purple red of *tabacum* var. *purpurea* (U. C. B. G. 25/06). The leaves are intermediate in

shape and texture, and they lack the distinctive constriction at the base so characteristic of the *tabacum* parent.

F_1 *sylvestris-tomentosa*, the third member of the series, is illustrated in plate 8, figure 1; its parents in plate 9. The *tomentosa* plant has been cut back and is therefore not wholly typical of the species. Flowers of *tomentosa*, and F_1 *sylvestris-tomentosa* are illustrated in figure 1. Flowers of the F_1 hybrid are pinkish in color, intermediate between the white of *sylvestris* and the salmon red of *tomentosa*. The F_1 hybrid is exceedingly vigorous, possibly even surpassing *tomentosa* in this respect; and its characters are mainly a synthesis of those of the two parents.

For the sake of comparison, measurements of flowers of the pertinent forms in the *sylvestris-tomentosa-tabacum* series are presented in table 1.

TABLE 1

FLOWER SIZE MEASUREMENTS IN THE *sylvestris-tomentosa-tabacum* SERIES

Type	Spread in mm.	Length in mm.
<i>tabacum</i> var. <i>purpurea</i>	36.0	49.3
<i>sylvestris</i>	42.5	85.3
<i>tomentosa</i>	30.0	31.0
<i>tomentosa</i> (including anthers).....	30.0	52.0
<i>tabacum</i> var. <i>purpurea</i> haplont.....	25.0	40.0
F_1 <i>sylvestris-tomentosa</i>	35.0	56.0
F_1 <i>tomentosa-tabacum</i>	27.0	34.0
F_1 <i>sylvestris-tabacum</i>	44.1	59.0

F_1 CYTOLOGY

Chromosomal behavior in F_1 *sylvestris-tabacum* has already been described in some detail (Goodspeed and Clausen, 1927b). The hybrid uniformly exhibits the Drosera scheme of conjugation, $12_{II} + 12_I$, in diaphase and I-M. The conjugants separate normally; the univalents are distributed for the most part at random in I and divide in II. The twelve bivalents of this hybrid are taken to represent conjugation of *sylvestris* with *tabacum* homologues in view of the prevailing absence of conjugation in *tabacum* haplont (Chipman and Goodspeed, 1927) and the chromosomal situation in backcross progenies of F_1 *sylvestris-tabacum* \times *sylvestris*, which are found to conform to the formula $12_{II} + i_I$, i_I exhibiting values from 0 to 12, with, however, a marked concentration of values in the lower and upper portions of

the range (Goodspeed and Clausen, 1927b). The F_1 hybrid has not given progeny from self-fertilization but from ten to twenty seeds per capsule are obtained by backcrossing it to its parental species, *sylvestris* and *tabacum*.

F_1 *tomentosa-tabacum* also exhibits the *Drosera* scheme, $12_{II} + 12_I$, in diaphase and I-M, with clear distinction between bivalents and univalents (figs. 2-4). The subsequent behavior, however, is somewhat different from that characteristic of F_1 *sylvestris-tabacum*. Separation of the conjugants is effected normally, but I-A (fig. 3) usually exhibits a number of univalents in advanced stages of division.

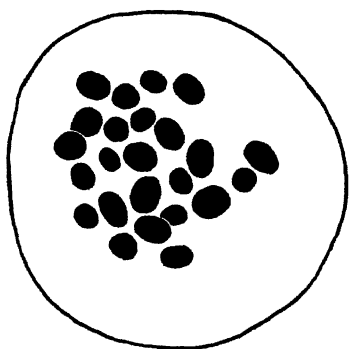


Fig. 2

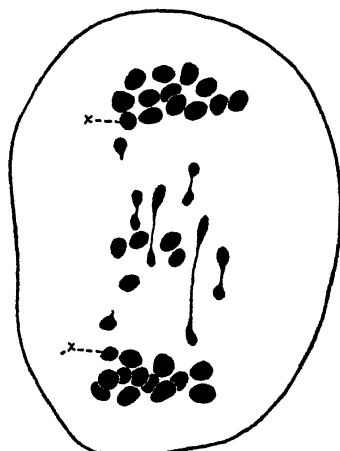


Fig. 3

Fig. 2. F_1 *tomentosa-tabacum*, I-M, $12_{II} + 12_I$.

Fig. 3. F_1 *tomentosa-tabacum*, I-A, illustrating division of univalents, x, x, halves of univalents.

In F_1 *sylvestris-tabacum* (Goodspeed and Clausen, 1927) and F_1 *paniculata-rustica* (Goodspeed, Clausen and Chipman, 1926) similar figures are also seen but in them it was shown that II-M plates rarely exhibit a total in excess of 36 chromosomes, which indicates that separation of the halves rarely is completed. In F_1 *tomentosa-tabacum*, however, II-M counts usually exhibit a total of more than 36 chromosomes. In 18 PMC in which both plates were countable, only three gave a total of 36 chromosomes and the average total per PMC was 40.4. Counts of 112 single II-M plates gave an average of 20.0 chromosomes per plate, which is substantially in agreement with the value obtained from PMC in which both plates were countable.

The subsequent behavior does not appear different from that described for F_1 *sylvestris-tabacum* and F_1 *paniculata-rustica*. Lagging in II-A was not conspicuous, but tetrad counts indicated the occurrence of a considerable amount of elimination and also the production of a small percentage of dyads. Two separate counts are included in table 2.

The F_1 *tomentosa-tabacum* hybrid exhibits a slight degree of fertility when *tabacum* pollen is applied to it, apparently approximately equal to that shown by F_1 *sylvestris-tabacum*. Trials with *tomentosa* have not been made.

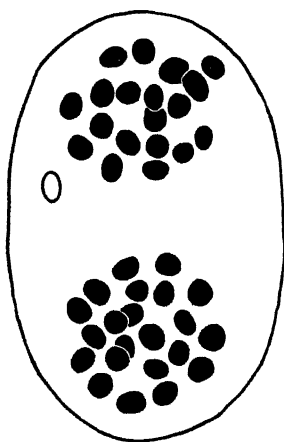


Fig. 4

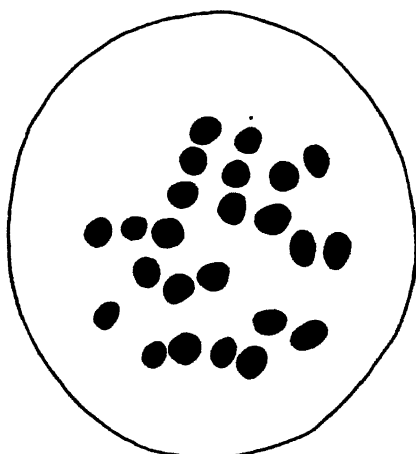


Fig. 5

Fig. 4. F_1 *tomentosa-tabacum*, II-M, 22 chromosomes in left plate, 19 in right plate, and one in the plasma.

Fig. 5. F_1 *sylvestris-tomentosa*, "I-M," with 24 unpaired chromosomes.

TABLE 2

TETRAD COUNTS IN THE *sylvestris-tomentosa-tabacum* SERIES

Hybrid	Types of pollen groups									Anomalous
	2 ₀	2 ₁	2 ₂	2 ₃	4 ₀	4 ₁	4 ₂	4 ₃	4 ₄	
F_1 <i>sylvestris-tabacum</i>	1	...	2	...	89	76	27	4	...	3 ₃
F_1 <i>tomentosa-tabacum</i>	82	67	42	8	1	
F_1 <i>tomentosa-tabacum</i>	3	...	1	...	55	62	33	14	1	3 ₀ , 3 ₀
F_1 <i>sylvestris-tomentosa</i>	45	10	283	4	1	3 ₀
<i>tabacum</i> haplont..... ..	8	9	6	4	111	27	7	9	1	3 ₀ (11), 3 ₁ (3), 3 ₂ (4)

F_1 *sylvestris-tomentosa* usually exhibits 24_1 at diaphase and I-M (fig. 5) although a small proportion of PMC in I-M and I-A may exhibit a few "bivalents" as in the *tabacum* haplont (Chipman and Goodspeed, 1927). In figure 7, I-A shows 7_1 approaching one pole, 9_1 near the other, 6_1 in the equatorial region and a "bivalent" disjoining. Sometimes as in figure 6 there is evidence of division of univalents. In this PMC, an extreme example, there were $11\frac{1}{2}$ near one pole, $6\frac{1}{2}$ near the other, and in the center three undivided univalents together with three which were undergoing division. Instances in which both I-M plates were countable, however, indicate that division of univalents in I is not characteristic of this hybrid.

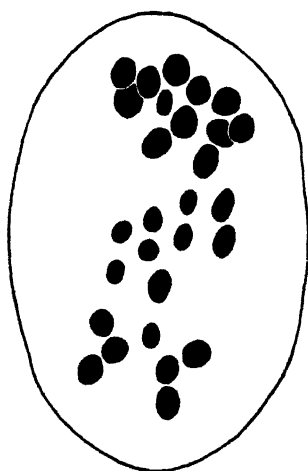


Fig. 6

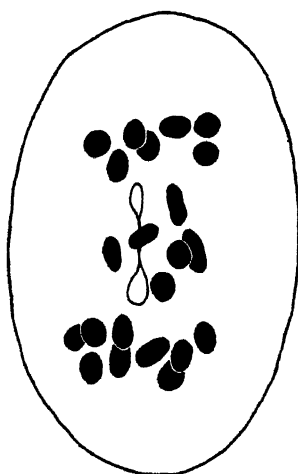


Fig. 7

Fig. 6. F_1 *sylvestris-tomentosa*, I-A, $11\frac{1}{2}$ at one pole, $6\frac{1}{2}$ at the other, 3_1 in the equatorial region, illustrating division of univalents.

Fig. 7. F_1 *sylvestris-tomentosa*, I-A, 7 at one pole, 9 at the other, 6 in the equatorial region and a "bivalent" (in outline) disjoining.

The counts of pollen groups contained in table 2 disclose the occurrence of a relatively large percentage of "restitution" divisions in this hybrid. The $2_0 + 2_1$ pollen groups amount to 16.0 per cent of the total number. Meiotic behavior leading to the production of dyads was not studied but a single record of a 0/24 distribution was presumably of this type. Dyad production is a not uncommon feature of *Nicotiana* hybrids, and while it appears to be most characteristic of hybrids in which the chromosomes fail to conjugate, it is also observed in those which exhibit the *Drosera* type of conjugation.

F₁ *sylvestris-tomentosa*, despite its conspicuous production of dyads, has as yet produced no seed either from selfing or from backcrossing to the parental species.

DISCUSSION

According to the data reported above and in previous papers, the F₁ *sylvestris-tabacum* and *tomentosa-tabacum* hybrids both exhibit 12_{II} + 12_I chromosomes in meiosis. In view of the fact that *sylvestris* and *tomentosa* are so distinctly different morphologically, and that *tabacum* haplont (Chipman and Goodspeed, 1927) and F₁ *sylvestris-tomentosa* typically exhibit no conjugation of chromosomes, it seems reasonable to assume that *tabacum* possesses two sets of chromosomes; one homologous with that of *sylvestris*, the other with that of *tomentosa*. Further data may be necessary to establish this conclusion: but at present it appears to be the only assumption consistent with all the facts outlined above.

Tabacum hybridizes readily with a number of other species. In addition to those described above we have studied its hybrids with the 12-chromosome species, *glauca* and *glutinosa*; but as yet our evidence is incomplete. Hybrids of *tabacum* with the 9-chromosome species, *alata*, the 12-chromosome species, *solanifolia*, and the 24-chromosome species, *Bigelovii* (Goodspeed and Clausen, 1927), all exhibit complete absence of pairing in F₁ meiosis. The hybrids of *tabacum* with *sylvestris* and *tomentosa* appear, therefore, to be unique as respects meiotic phenomena; and in addition, they are the only partially fertile ones in the series. Brieger (1928) has, however, reported a hybrid of *tabacum* with the 12-chromosome species, *Rusbyi*. He finds that this hybrid exhibits the Drosera scheme of conjugation, and also that it produces some offspring when backcrossed to *tabacum*. We have *Rusbyi* in our collection, and find that it is clearly a species closely allied to *tomentosa*. It is not surprising, therefore, that it exhibits parallel behavior in crosses with *tabacum*.

Since *tabacum* possesses chromosome sets homologous with *sylvestris* and *tomentosa* it may be assumed that its progenitor arose through hybridization of two species, progenitors or close allies of *sylvestris* and *tabacum*, followed by doubling of chromosome number after the manner described for the origin of *digluta* (Clausen and Goodspeed, 1925; Clausen, 1928) by doubling of the chromosome number in a *glutinosa-tabacum* hybrid. If this assumption is correct, F₁ *sylvestris-*

tomentosa should bear a general morphological resemblance to *tabacum*. To a certain extent this is the case, but a considerable enlargement of floral and vegetative organs and certain structural differences preclude an appeal to these species as they now exist as the immediate ancestors of *tabacum*. Moreover, our studies of inheritance in the *sylvestris-tabacum* hybrid indicate the existence of extensive genetic differences between homologous chromosomes of these two species. It is probable that *tabacum* has undergone evolutionary changes since establishment of its chromosome number, and that its putative ancestors have also been modified; but the cytological evidence presented above indicates that these subsequent changes have not been sufficient to destroy the affinity between their chromosomes. It seems that further studies by the method here outlined may lead eventually to production, as Jorgensen (1928) suggests, of an existing species from hybridization of two other species.

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EXPLANATION OF PLATES

PLATE 8

Fig. 1. F_1 *sylvestris-tomentosa*.

Fig. 2. On the left, a plant of F_1 *tomentosa-tabacum*; on the right a haplont *tabacum* which occurred in the same F_1 population.



PLATE 9

Fig. 1. *N. sylvestris*.

Fig. 2. *N. tomentosa*, three years old, somewhat cut back.



Fig. 1



CHROMOSOME NUMBER AND MORPHOLOGY
IN NICOTIANA

III. THE SOMATIC CHROMOSOMES OF
N. LONGIFLORA CAV.

BY
LILLIAN HOLLINGSHEAD

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CHROMOSOME NUMBER AND MORPHOLOGY IN NICOTIANA

III. THE SOMATIC CHROMOSOMES OF *N. LONGIFLORA* CAV.

BY
LILLIAN HOLLINGSHEAD

INTRODUCTION

In an effort to present evidence as to the origins and relationships of certain species of the genus *Nicotiana*, a variety of studies have been carried on in the University of California Botanical Garden during the past twenty years. In addition to Mendelian studies and investigations of chromosome homology in interspecific hybrids, determinations of chromosome number and morphology are being made.

Three articles in this series have already appeared (Ruttle, 1927, 1928; Goodspeed and Avery, 1929). Together with the data included herein and the results of similar unpublished investigations on other species, they indicate that characteristic distinctions in chromosome morphology as well as in chromosome number distinguish certain species of *Nicotiana*.

The Petunioides section of the genus is very loosely constituted from the genetic point of view (cf. East, 1928, p. 308). Within it a number of more or less well defined species groupings appear to exist and further investigation may make possible their genetic and taxonomic delimitation. Chromosome studies of various sorts have, for some time, been carried on among certain species included in this section—*alata*, *longiflora*, *sylvestris*, *Bigelovii*, and *acuminata*—in the hope of obtaining pertinent data in this connection.

N. longiflora is a somewhat variable species but probably is not so definitely polymorphic as are the species *tabacum*, *rustica*, and *Bigelovii*. It is rather well known in horticultural practice and as a weed in warmer climates (Setchell, 1912). It has often been described and figured (cf. Setchell, *loc. cit.*, East, 1916). As grown from various sources in the U. C. B. G. it has exhibited considerable variation in flower size and, to a lesser degree, in vegetative characters.

N. plumbaginifolia Viv. according to East (1928) is a member of the *longiflora* group and does not deserve specific designation, and the evidence which we have obtained supports this conclusion. Reported hybrids between members of the *longiflora* group and other *Nicotiana* species are restricted to the *alata* aggregation, *tabacum*, and *suaveolens* (cf. East, *loc. cit.*, pp. 268-269, Malloch and Malloch, 1924).

MATERIALS AND METHODS

Root tips from seedlings were examined and the effects of the following fixing agents were compared.

(1) Chrom-acetic-formalin

1 part

65 cc water

10 cc glacial acetic acid

1 gr. chromic acid

1 part

40 cc formalin

35 cc water

(2) Weak Flemming's solution (Chamberlain, 1924)

(3) Chrom-acetic-formalin

45 cc 1 per cent chromic acid

3 cc glacial acetic acid

12 cc formalin

This solution to act over night followed by 1 per cent chromic acid for several days.

Clearlest somatic chromosome plates were given by fixation (1) above and all but one of the figures included in what follows were drawn from material so fixed. Sections were stained with Haidenhain's iron-alum-haematoxylin exclusively.

CYTOLOGICAL OBSERVATIONS

The chromosome number of *longiflora* was determined in P. M. C. as $n=10$ by Goodspeed (1923). Meiotic counts of *longiflora* and mitotic counts of *plumbaginifolia* by Christoff (1928) confirm the original one. That 20 is the characteristic somatic number in this species was determined in this investigation, in the course of which an unusually large number of plates was found where the chromosome number was perfectly clear. The readiness with which such counts could be made is a product of a characteristic type of chromosome arrangement at the metaphase, apparently peculiar to this species.

As shown in figure 1, polar views of the metaphase exhibit the majority of the chromosomes lying in a horizontal plane, arranged like the spokes of a wheel. The remaining chromosomes, in the center, tend to lie somewhat at an angle with the major plate; at times the cut gives one or more of them the appearance of circular dots. Their characteristic spoke-like arrangement is suggested in Christoff's (*loc. cit.*) figure of *plumbaginifolia*.

The chromosomes as a group are relatively large although in total volume somewhat less than that of the chromosome garniture of *alata*, and none of the *longiflora* chromosomes is as long as the two longest pairs in *alata* (cf. Ruttle, *loc. cit.*, fig. 1). However, there does not

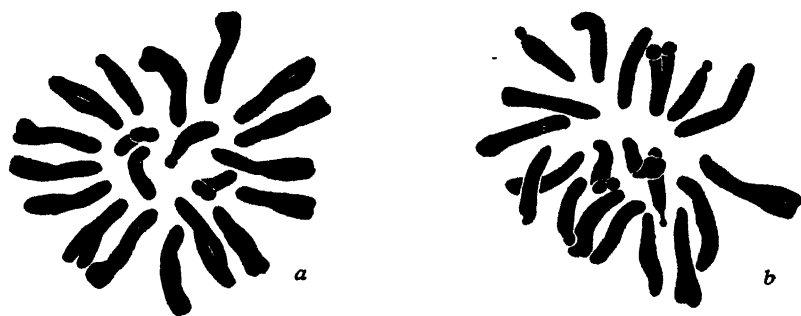


Fig. 1. Somatic metaphase plates of *Nicotiana longiflora*. *a*, characteristic chromosome arrangement in polar view. *b*, showing three constricted short chromosomes, $\times 3750$.

appear to be any such distinction in chromosome size between these two species as is shown in Christoff's figures (*loc. cit.*, pl. 1, figs. 13 and 14). Length differences within the *longiflora* set are shown in figure 1 but, with the exception of two pairs of rather distinctly shorter chromosomes, they are too slight to supply a basis for classification. After prolonged efforts, involving various fixatives, no evidence of the presence of satellites was found and only 4 of the 20 chromosomes clearly showed constrictions. As distinguished from most large or medium sized plant chromosomes, they never assume a V- or J-shape, although at times they may be slightly bent.

As employed by Taylor (1925*a* and *b*, 1926) the terms "proximal" and "distal" distinguish the two ends of the chromosome, the former referring to that at which spindle-fiber attachment occurs. Thus at metaphase the proximal end would in general be directed toward the center of the equatorial plate and at anaphase toward the pole. According to these definitions the four shorter chromosomes of *longiflora* which exhibit distinctive morphology are characterized by distal

constrictions. The metaphase evidence shown in figures 1, 2, and 3 indicates that the constricted ends are not usually oriented toward the center of the plate. Figure 3, *d*, shows them directed away from the poles at anaphase.

Such distal constrictions have been described by Taylor (*loc. cit.*) among others. Variations in the appearance of that end of the chromosome bearing a constriction are also noted by Taylor who assigned them primarily to distinctions in fixation. Thus, in *Fritil-*

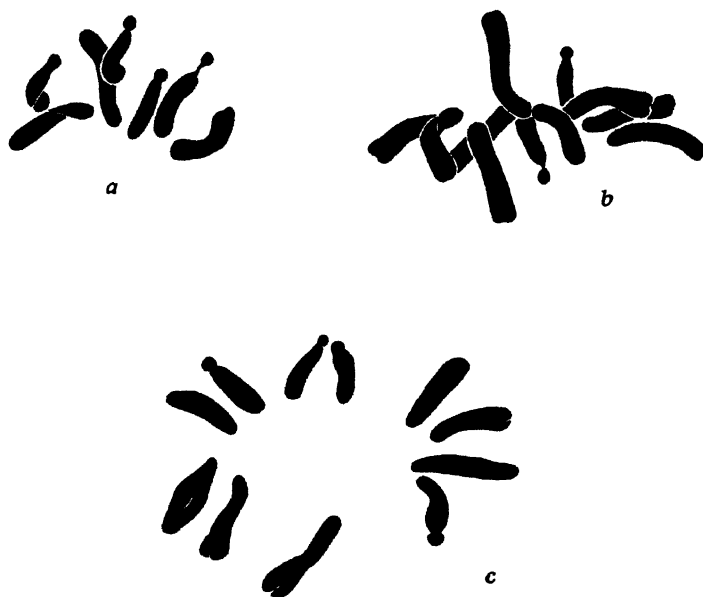


Fig. 2. *a* and *b*, lateral views of portions of metaphases to show constricted chromosomes. *c*, portion of polar view showing four constricted chromosomes, $\times 3750$.

laria imperialis, he describes a chromosome in which the distally constricted region may appear as a satellite attached by a thread to the main shaft. In figure 3, *a*, are included some of the variations in appearance which constricted areas of *longiflora* chromosomes may exhibit. Although they may correspond simply to fixation effects, it is not out of the question that they represent a partial expression of the range of variation in form characteristic of constricted chromosome areas in general.

It is clear that in many species a constriction or other similar structural modification occurs at the point of spindle fiber attachment. Indeed Navashin (1926) holds that an actual zone of greater or lesser

constriction is always present at such point and can be demonstrated by a suitable technique. Taylor (1926) states that he has "failed to find a case among any of the medium or large chromosome types studied in which a chromosome has a truly terminal fiber attachment." Heitz (1928) has recently emphasized his belief that every chromosome is fundamentally two armed. With these conclusions in mind efforts were made to demonstrate the presence of proximal fiber constrictions on the chromosomes of *longiflora*, it being from the start obvious that fiber attachment was close to the end if not actually terminal.



Fig. 3. *a*, various forms assumed by constricted chromosomes at metaphase. *b* and *c*, separation of early anaphase chromosomes in two different cells. The lines indicate the general direction of the spindle fibers. *d*, lateral view of portion of late anaphase to show rod-like shape of chromosomes, $\times 2867$.

Material from fixations (2) and (3), above, was used to supplement that from (1) since with other species they were known to enhance the definition of constrictions. No evidence could, however, be obtained to indicate the occurrence of proximal constrictions. Lateral views of anaphases further suggested that the fiber attachment of certain of the long chromosomes may be terminal. The appearance of chromosome halves in early anaphase is illustrated in figure 3, *b* and *c*. The proximal ends move apart in a fashion corresponding to terminal fiber attachment and the picture is somewhat different from that figured (cf. Taylor, 1926) for chromosomes with closely subterminal attachment. It was not possible to determine the

manner of separation for more than a few chromosomes in any one anaphase but this mode of separation was seen frequently.

Confirming the suggestion that spindle fiber attachment may in certain cases be terminal is the evidence from lateral views of late anaphase stages. A portion of an anaphase is shown in figure 3, *d*, which has already been referred to in connection with the occurrence of distal constrictions. The chromosomes are clearly quite straight and exhibit no sign of a reflexed area or even of the slightly "hooked" condition figured by Taylor (*loc. cit.*) at a corresponding stage for chromosomes with subterminal fiber attachments. Many late anaphases were studied but without exception the proximal portions of the chromosomes were smooth in contour and with no sign of hooks or bends. It cannot be denied, of course, that in these chromosomes the attachment may be slightly subterminal but in such case the question of distinction between the two positions is largely an academic one.

Some study was made of a smaller flowered race grown under the designation *N. longiflora* var. *purviflora*. As was to be anticipated no distinctions in somatic chromosome morphology between this variety and "typical" *longiflora* could be detected. A culture from open pollinated seed of *longiflora* also was grown and somatic plates from three plants were examined. The total number of chromosomes in each case was 19 and the presence of satellited and V-shaped units typical of *alata* (cf. Ruttle, *loc. cit.*) was readily demonstrable. The number of chromosomes (*alata*, $n=9$) and their morphology thus indicated that these plants were F_1 hybrids of *alata* and *longiflora*.

A preliminary examination of chromosome number and behavior in P. M. C.'s of *longiflora* was attempted, using both aceto-carmin and paraffin preparations. At I-M 10 bivalents were usually seen but, rarely, 11 units occurred. In such cases one or two chromosomes seemed paler in color than the others. At times there appeared to be lagging chromosomes at the anaphases. At diaphase some variation as to pairing was occasionally seen; ten well formed pairs was the rule but P. M. C.'s occurred in which conjugation was loose in the case of one or two pairs. Other workers in this laboratory have, from time to time, noted a considerable incidence of 9-11 II-M P. M. C.'s in *longiflora*. The fragmentary evidence on meiotic stages noted above suggests that such distributions may follow a tendency to non-conjunction as well as being, possibly, the result of non-disjunction.

By way of summary it may be said that the somatic chromosome garniture of *N. longiflora* consists of 20 units differing somewhat in

length. Two pairs, the shorter, generally bear distal heads, separated from the shaft of the chromosome by more or less definitely marked constrictions, and there is evidence that spindle fiber attachment of certain chromosomes, at least, may be terminal in position. General and to some extent specific distinctions in morphology definitely separate portions of the *longiflora* chromosome complex from portions of the *alata* complex. Its distinction from other members of the *Petunioides* section of the genus also appears to be definite but on this point further evidence is still needed.

The writer is much indebted to Dr. T. H. Goodspeed, under whose direction the investigation was carried out, for the material and for helpful advice throughout the course of the work.

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CHROMOSOME NUMBER AND MORPHOLOGY
IN NICOTIANA

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BY
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INTRODUCTION

Nicotiana alata Lk. et Otto has been the subject of considerable genetic and cytological investigation from the time of Naudin, Godron, and Focke. It is rather widely employed horticulturally, usually under the designation *N. affinis* Moore. The species is included in the Petuniodes section of the genus, together with other species having salver-shaped corollas which are tinged with red or purple, and are arranged in racemes or panicles. As suggested by East (1928, p. 308), there are in this section "a number of genetic centers" and it would appear that the species *alata*, *Langsdorffii*, and *Sanderae*, all of which possess 9_{II} and give fertile hybrids on crossing, form one such center. A number of varieties of *N. alata* have been described but the varietal distinctions noted seem to be no greater than the extremes of variation in any given strain.

Genetic investigation in the case of *alata* has been concerned chiefly with character analyses in crosses with other species. Thus East (1913, 1916) studied the inheritance of flower size in hybrids between *N. Langsdorffii* and *N. alata* and between *N. Forgetiana* and *N. alata* var. *grandiflora* Comes. East and Park (1917) have also analyzed the genetic basis for the self-sterility characteristic of most strains of *alata*.

The chromosomes of *alata* have been the subject of some recent cytological investigation. Goodspeed (1923) was unable, with the material at the time available, to decide whether 8, 9, or 10 pairs were characteristic for *alata*. Christoff (1928) reports 8 pairs for *alata*, 16 chromosomes being found in somatic cells, and 8 at I-M and II-M.

Vilmorin and Simonet (1927) found $n=9$ to be characteristic of P.M.C. of *alata*. Ruttle (1927) has shown that 18 somatic chromosomes uniformly occur in cultures of this species grown in the University of California Botanical Garden and that a considerable number of pairs exhibit distinctive chromosome morphology.

Ruttle also found 9_{II} at I-M, but at II-M many P.M.C. showing metaphases, one with 8 and another with 10 chromosomes. This condition was assumed to be the reflection of a considerable incidence of non-disjunction and, unless the $n-1$ and $n+1$ pollen grains were inviable or produced non-viable zygotic combinations, monosomic and trisomic individuals should occur rather frequently in *alata*. It seemed probable that a portion of the variation observed in some of our cultures of this species might be dependent upon such altered chromosome contents. This point has been under investigation during the past two years.

Since $n-1$ and $n+1$ pollen grains may compete unsuccessfully with normal pollen, controlled pollination was employed and, in a population of 80 plants from such pollination, one monosomic plant was obtained. Like normal individuals of most strains of *alata* this plant was self-sterile at the height of its blooming, but gave seed readily with sister 18-chromosomed plants. The progenies from these crosses furnished abundant material, in which it was thought that it might be possible to detect the morphological influence of extra or lacking chromosomes and to study the transmission of the unpaired chromosome. Somatic plates in root tips were used to determine chromosome number in the progenies mentioned, P.M.C. being used to check these counts in some cases, and for the study of chromosome distribution in meiosis of chromosomal variants.

METHODS

All root tips were killed and fixed in chrom-acetic-formalin, the formula used being the same as No. 1 mentioned by Hollingshead (1929). This formula was also used with anthers, but best results were obtained when a one-half minute's treatment with Carnoy's fluid preceded the chrom-acetic-formalin.

Paraffin sections were cut 8μ thick for root tips and 10μ for anthers. All slides were stained in Heidenhain's iron-alum haematoxylin. For P.M.C., aceto-carmin smears proved most satisfactory but some use was also made of permanent smears made according to Webber (1929a).

SOMATIC CHROMOSOMES

NUMBER AND MORPHOLOGY

From the monosomic plant mentioned above, two populations totaling 79 plants have been grown and examined cytologically. In these populations the constant recurrence of the 18 chromosomes characteristic of the species was readily determined. For the plants with 18 chromosomes the general classification of chromosomal types given by Ruttle was verified. Four long, usually V-shaped chromo-

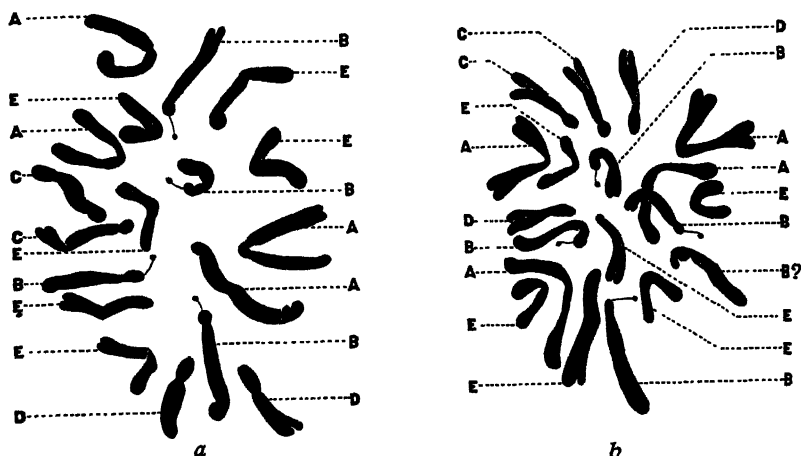


Fig. 1. *a*, *Nicotiana alata* var. *grandiflora*. Diploid somatic plate showing the following chromosome complement: *A*, 2 pairs, long, with median constriction; *B*, 2 pairs, medium, with subterminal constriction and proximal satellite; *C*, 1 pair, medium, with subterminal constriction; *D*, 1 pair, slightly shorter, with subterminal constriction; *E*, 3 pairs, medium, with median or submedian constriction. *b*, somatic plate of *alata* trisomic. The nineteenth chromosome marked *B?* is probably a satellited one. $\times 3400$.

somes with median construction are indistinguishable from each other and form a well marked group 1. In figure 1*a* these chromosomes are marked *A*. Ruttle's group 2 contained a single pair of satellited chromosomes, but, as she suggested, it should contain two such pairs. In the present study, at least 32 plates showed four satellited chromosomes although plates with two and three satellited chromosomes were the most frequent. In plates in which all four satellited chromosomes are lying flat and show well marked constrictions, the latter are subterminal, forming a small head to the proximal end of the chromosome to which the satellite is attached. Often these chromosomes are bent

or somewhat V-shaped or assume a nearly vertical position in the center of the plate so that the point of constriction is not apparent. However, in the best plates the four satellited chromosomes appear to be identical, and form an easily distinguished group 2. In figure 1a these chromosomes are marked *B*.

The five remaining pairs of chromosomes belong to Ruttle's group 3. A further subdivision of this group seems possible on the basis of chromosome constrictions. One pair is rather similar in length to the satellited pairs, and like them has a subterminal constriction forming a small head. This pair is marked *C* in figure 1a. Another pair is somewhat shorter with a subterminal constriction a little farther from the end of the chromosome and is designated *D* in figure 1a. The other three pairs are all intermediate though somewhat varying in length, and have median or submedian constrictions. Five of these six chromosomes were shown in figures 1a and 4b of Ruttle's paper. In figure 1a all these six chromosomes are marked *E*.

A recent accession of *N. alata* from Uruguay (Herter No. 82891) has been grown, and somatic plates examined in the root tips show the same chromosome number and morphology as in the 18-chromosomed plants of the strain of *N. alata* var. *grandiflora* used in the present study.

With these morphological distinctions in mind it becomes possible to recognize at least some of the chromosome types duplicated or missing in plants with deviating chromosome number. Of the 57 plants examined where the monosomic was the male parent, 55 plants showed 18 somatic chromosomes and 2 showed 19. In both these simple trisomic plants the additional chromosome is one with a subterminal constriction, and possibly in each case it is a member of the satellited group. Thus in figure 1b there are four chromosomes bearing distinct satellites, and, in addition to the normal complement of chromosome types, one chromosome marked *B*? with a subterminal constriction and what appears to be a satellite close to the proximal end of the chromosome without the usual rather long thread of attachment.

Of the 22 plants where the monosomic was the female parent, 18 proved to possess the normal 18 (one also had 36) while four showed more than 18 chromosomes, but none corresponded to the simple trisomic types found in the reciprocal progeny. The divergent chromosome numbers were 22, 25, 26, and 27.

In the case of the plant with 22 chromosomes, somatic plates showed that there had been an addition of two long V-shaped (*A*) chromosomes to the normal two pairs of this type. Thus in figure 2*a* six instead of the usual four chromosomes are marked *A*. The remaining two additional chromosomes belong to the group of chromosomes of intermediate length.

In somatic plates of the plant which showed 25 chromosomes there could be distinguished 6 large V-shaped (*A*) chromosomes, 5 satellited (*B*) chromosomes, at least 9 of the chromosomes of intermediate length with median constriction (*E*), leaving 5 with subterminal con-

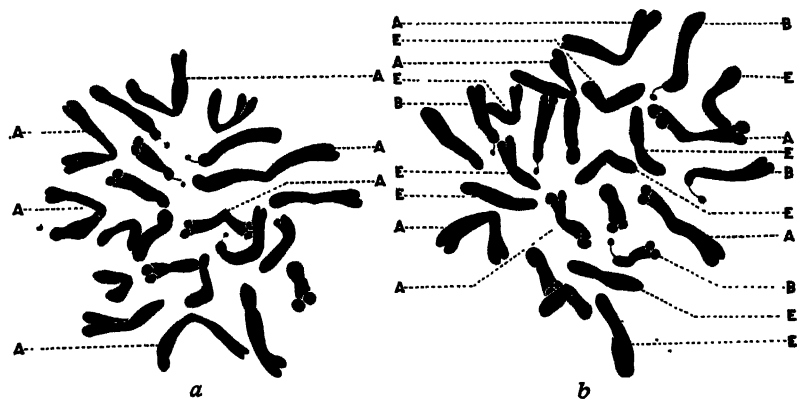


Fig. 2. *a*, somatic plate showing 22 chromosomes, 6 of which are long with median constriction (*A*). *b*, somatic plate showing 25 chromosomes. The two chromosomes missing from the full triploid complement are chromosomes with subterminal constrictions, one of which may be satellited. $\times 3400$.

strictions (*C* and *D*). The two chromosomes missing from the full triploid complement of 27 are apparently chromosomes with subterminal constrictions, of which one may be satellited. Thus the deficiency appears to be in the same group of chromosomes found to be duplicated in the trisomics (fig. 3*b*). In the plant with 26 chromosomes, 6 large V-shaped and 5 satellited chromosomes were seen, but the one chromosome missing from the full triploid set could not be identified. In the plant with 27 chromosomes, a full triploid set could be distinguished, there being 6 of the large V-shaped (*A*) chromosomes, at least 5 of the satellited chromosomes (*B*), 9 of intermediate length with median constrictions (*E*), and 7 with subterminal constrictions (*C* and *D*), of which one probably was satellited (fig. 3*a*).

Three root tips of one plant were found to be completely tetraploid, but that it was not a true tetraploid plant was shown by counts of 9_{II} at I-M in the P.M.C. and the occurrence of one diploid root tip.

In the tetraploid somatic metaphases 8 of the large V-shaped (*A*) chromosomes and at least 6 of the satellited (*B*) chromosomes could be distinguished. In addition, there were 12 chromosomes of intermediate length with median constrictions (*E*) and 10 with sub-terminal constrictions of which 2 were no doubt satellited. Here, then, a doubling of the normal somatic set of 18 chromosomes had occurred (fig. 3*b*) possibly by a process involving nuclear division without cell division in a manner similar to that described for the origin of tetraploid sectors in roots of other species (Hollingshead, 1928, Lesley, 1925).

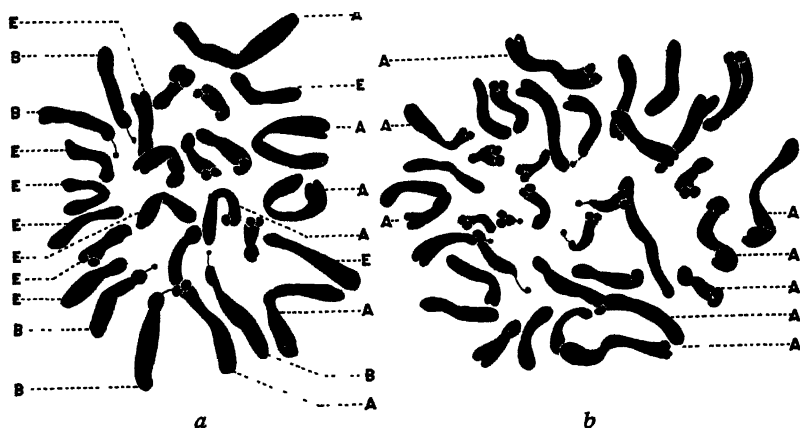


Fig. 3. *a*, somatic plate of triploid *alata*. Here all except one satellited chromosome of the full triploid set can be identified. *b*, somatic plate from a tetraploid root of a normal diploid plant. The 8 long chromosomes with medium constrictions are marked *A*, and 6 of the 8 satellited chromosomes can be identified. $\times 3400$.

EXTERNAL MORPHOLOGY OF CHROMOSOMAL VARIANTS

The loss or addition of a single chromosome is apparently reflected in little, if any, alteration in external morphology of *alata*. The failure to secure distinct trisomic and monosomic types here may in part be due to the polymorphic nature of this species. Thus the two simple trisomies showed no obvious variation in external morphology as compared with sister plants which contained the normal 18 chromosomes. The original monosomic was less vigorous but was otherwise indistinguishable from normal. This is in contrast to the situation in *N. tabacum* where at least four different monosomic and trisomic types are distinguished by obvious character alterations.

The plant with 22 chromosomes was, however, a striking variant throughout the season, being a dwarf plant with small thick leaves and flowers which were small, split, or otherwise deformed. It produced almost no pollen, and only a few viable eggs. The pronounced effect of the additional chromosomes in this case may be due to the number of chromosomes added (4) which form an unbalanced set, or it may be that the duplication of the long V-shaped chromosome has a greater effect than the addition of other chromosomal types.

The plant which showed 25 somatic chromosomes was abnormal in appearance early in the season, producing misshapen split flowers. Late in the season, however, the flowers were more normal in appearance. The triploid plants with 26 and 27 chromosomes showed no striking variations in external morphology as compared with normal 18-chromosomed individuals. Thus the addition of a complete or nearly complete haploid set produced no changes in external morphology, while the addition of seven chromosomes resulted in significant alteration, and the addition of four produced a highly abnormal plant.

In these two populations pistillody of the stamens was found to occur in certain plants. The expression of this peculiar character varied from flowers in which the apices of the anthers bore a single small stigma to those in which the anther was almost completely metamorphosed, one or more well developed styles and stigmas and ovules or their rudiments occurring. In some cases the general configuration of the corolla was altered also. This character is apparently a simple Mendelian recessive, for a fairly good agreement with a 3:1 ratio was evident in each of the populations, and an even better agreement when the two populations were combined. Thus in one population there were 10 plants with stigmatoid anthers and 28 without, and in the other, 9 with stigmatoid anthers and 33 without, making a total of 80 plants of which 19 showed the recessive character.

Apparently both of the parents used in the cross were heterozygous for this recessive character. Undoubtedly it has been present in strains of *alata* for considerable time, although no account of a genetic analysis has been found. Costerus in 1907 reported an anatomical study of pistillody of the stamens occurring in a single *N. affinis* (*alata*) plant. Further genetic study of the stigmatoid anther character in *alata* is being carried on.

MEIOSES. P.M.C.

Examination of meiotic stages particularly in the case of plants with deviating chromosome numbers, gave a corroboration of the chromosome situation determined from root tips. 9_{II} at I-M were found in plants investigated where counts of 18 were obtained in

TABLE 1

CHROMOSOME BEHAVIOR IN AN *Nicotiana alata* TRISOMIC

a. Chromosomes off the plate at I-M, side view								
Number of chromosomes	0	1	2	3	4	5	Total	
Number of P. M. C.....	60	60	38	23	6	3	190	

b. Chromosomes in plasma at interkinesis and II-M									
Number of chromosomes	0	1	¹ divided	2	² divided	3	4	5	Total
Number of P. M. C...	409	69	35	25	11	18	3	1	571

c. II-M counts															
Number of chromosomes	^{*7} 0	^{*7} ₀ ³	⁸ 0	⁸ ₀ ¹	^{*8} 11	⁹ 0	⁹ ₀ ¹	⁹ 9 ¹	⁹ 10	⁹ 11	¹⁰ 0	¹⁰ ₀ ¹	¹⁰ 11	¹¹ 0	Total
Number of P.M.C....	2	1	12	1	3	45	8	3	20	2	40	1	1	4	149

d. Tetrad counts									
Number of cells	2	2+1	2+2	3	3+1	4	4+1	4+2	Total
Number of P. M. C...	41	26	1	2	2	448	46	1	567

$\frac{*7}{0}$ represents a P. M. C. in which only one II-M plate was countable; $\frac{7}{0^3}$ indicates that only one plate was countable and three chromosomes were seen in the plasma; $\frac{8}{11}$ represents a P. M. C. in which both plates were countable.

somatic cells, and the total number of chromosomes at I-A corresponded to the somatic number in plants which showed deviating chromosome numbers in the root tips.

Certain apparently significant points were brought to light in these P.M.C. studies. In the first place it was clear that chromosome multiples larger than two do not ordinarily occur at I-M in *N. alata*, as is also the case with *N. tabacum* and *N. rustica*. Thus trisomic

plants did not show $8_{II} + 1_{III}$ nor did the triploid plant show 9_{III} , and, although an apparent trivalent was now and then observed, additional chromosomes behaved, in general, as univalents at the first meiotic division.

In the second place, a considerable incidence of non-conjunction was characteristic of the trisomic plants. Whereas, as already indicated, 9 or 10 units at I-M should be expected in such a plant, P.M.C. with 11 or 12 units were most frequent, 3 to 5 of them representing univalents as indicated by their size and shape (figs. 4a, b, c). Correspondingly, lateral views of I-M which according to expectation should not exhibit more than one chromosome off the equatorial plate, showed from 2 to 5 so located in 37 per cent of 190 P.M.C. (cf. table 1, a). At interkinesis and II-M, over 10 per cent of 571 P.M.C. showed from 2 to 5 chromosomes in the plasma in addition to over 18 per cent that showed a single chromosome lagging or dividing in the plasma (cf. table 1, b). Chromosome counts at II-M (cf. table 1, c) corroborated the I-M evidence that non-conjunction had occurred, in that only 70 per cent of single plates contained 9 or 10 chromosomes, the remaining 30 per cent showing chromosomes in the plasma or more or less than 9 or 10 chromosomes in the II-M plates (fig. 4, d, e, f).

The lack of conjugation seen within the normal set of 18 chromosomes in the trisomic seems to be greater than that occurring in normal 18-chromosomed plants. It may be that an unbalanced chromosome set tends to increase a tendency, perhaps inherent, to non-conjunction. Lesley (1928) found that trisomic tomato plants of one type occasionally gave rise to trisomics of other chromosomal constitution, and assigned this behavior to irregularities of bivalents which under other conditions conjoined and disjoined normally. Beadle and McClintock (1928) found that male sterility in *Zea Mays* is due to a recessive Mendelian factor causing irregular meioses and producing a partial or complete failure of synapsis. This suggests that the addition of a chromosome carrying certain genes might be responsible for non-conjunction such as that seen in the *alata* trisomics.

A third observation of significance in these P.M.C. studies was the frequent occurrence of diploid metaphases in a trisomic plant. About the periphery of anther sacs filled with normal second division stages, cells showing 19 units on a large spindle occurred (fig. 5a). The chromosomes divided to give dyads which were present to the extent of 12 per cent at the tetrad state (cf. table 1, d).



Fig. 4. P.M.C. of *alata* trisomic. *a*, I-M, $9u+1v$. *b*, early I-M, $9u+3v$. *c*, I-M, $7u+5v$. *d*, most frequent II-M distribution (9-10). *e*, II-M, 9-1-9, showing the extra chromosome between the two plates of nine each. *f*, 8-11 distribution at II-M. $\times 2500$.

Some evidence as to the possible mode of origin of these diploid metaphases was secured. Although it may be that in such P.M.C. the chromosomes form a metaphase plate without pairing and then undergo division in a way similar to that described by Belling (1925) for *Uvularia*, it seems more probable that a metaphase stage has preceded the formation of these plates, perhaps a semi-heterotypic division similar to that described by Rosenberg (1926-27). In one anther sac filled with normal binucleate interkineses, P.M.C. with a single nucleus occurred, the chromosomes numbering 19, and corresponding in structure to normal interkinetic chromosomes (fig. 5*b*).

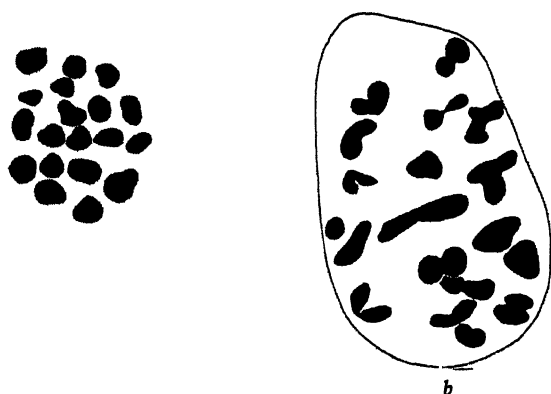


Fig. 5. *a*, diploid metaphase in *alata* trisomic. 19 chromosomes in one large spindle. *b*, diploid interkinesis in *alata* trisomic. $\times 2500$.

Again in some P.M.C. a dumbbell-shaped nucleus was seen, suggesting that a heterotypic anaphase had been arrested. Occasionally these metaphases showed 18 chromosomes in a single plate with the nineteenth chromosome above or below the plate. This suggests that some previous stage had resulted in the wandering of the unpaired chromosome.

There is evidence that diploid meioses of the type just described for a trisomic also occurred in the original monosomic which was a parent of the progenies under discussion. On this basis the origin of the plants with divergent chromosome numbers may be suggested. Since triploids were found only in the population in which the monosomic was the female parent, it may be assumed that all the functional diploid gametes were megaspores produced by the monosomic. In this case, the 27-chromosomed plant must have been produced by the union of a 17-chromosomed egg and an $n + 1$ gamete from the normal parent.

Likewise the 25-chromosomed plant probably originated from the fertilization of a 17-chromosomed egg by an $n-1$ pollen grain. It therefore represents a doubly modified triploid. The 26-chromosomed plant no doubt arose from the union of a 17-chromosomed egg and a normal 9-chromosomed pollen grain. The two trisomies among the 57 plants from the cross of normal \times monosomic probably owe their origin to non-disjunction occurring in the E.M.C. of the normal plant. In this case all functional male gametes of the monosomic must have been normal as to chromosome number.

The apparent functioning of $n+1$ and $n-1$ pollen grains with diploid megaspores, giving rise to the plants with 25 and 27 chromo-

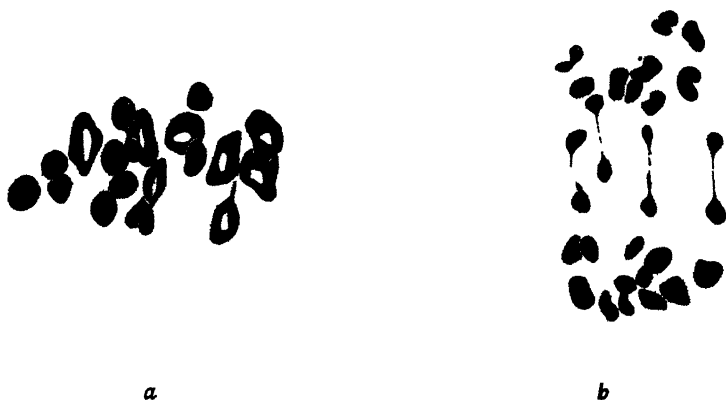


Fig. 6. *a*, I-M in plant with 26 chromosomes, $9n+8x$. *b*, I-T in plant with 26 chromosomes, 4 univalents lagging and apparently dividing. $\times 2500$.

somes when such pollen grains did not fertilize normal haploid megaspores, may be explained on the assumption that the diploid metaphases occur at a rather late stage and megaspores arising from this division may thus be late in developing an embryo sac. Such embryo sacs would then be available to the slower growing $n+1$ and $n-1$ pollen tubes or, in other words, a corresponding difference in growth and development may favor the union of two altered sets of gametes.

Chromosome behavior observed in meiotic stages of two triploid plants may be briefly mentioned. In the plant with 26 somatic chromosomes I-M usually showed $9n$ and $8x$ (fig. 6*a*), but rarely plates with less than a total of 17 units were found. This indicates an occasional formation of trivalent chromosomes. At the first anaphase

from 2 to the full 8 unpaired chromosomes lag on the spindle, part of these apparently undergoing division (fig. 6*b*). Usually the lagging chromosomes do not complete their division at this time, but II-M evidence indicates that occasionally division of univalents is actually

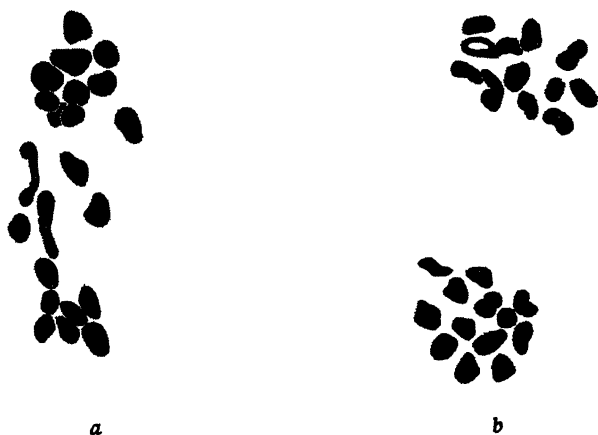


Fig. 7. *a*, I-T in plant with 25 chromosomes, 7 chromosomes lagging. *b*, II-M in plant with 25 chromosomes, 12-13 distribution. $\times 2500$.

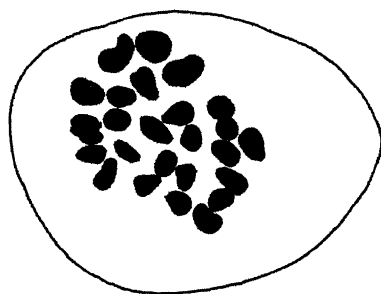


Fig. 8. Diploid metaphase in plant with 25 chromosomes. $\times 2500$.

accomplished. Lagging chromosomes were also seen in the second anaphase and these resulted in the formation of microcytes which were present in 21 per cent of the tetrads. Diploid metaphases were seen in a few cases and 6 per cent dyads were present at the tetrad stage.

In the plant with 25 chromosomes the chromosome behavior in meiosis was similar to that described for the 26-chromosomed plant.

At I-M, $9_{II} + 7_I$ were usually seen although rarely up to three trivalents were formed. Considerable lagging was the rule at I-A, all seven univalents sometimes being scattered between the two anaphase or telophase groups (fig. 7a). The distribution of chromosomes observed at II-M varied from 12-13 (fig. 7b) to 10-15 and 8-17. Here again a metaphase was occasionally seen with the full diploid number of chromosomes oriented on a single spindle (fig. 8). At the tetrad stage 6 per cent dyads and 13 per cent microcytes were present.

DISCUSSION

That the normal somatic chromosome number is 18 (cf. Ruttle, *loc. cit.*) was confirmed by the present study of the chromosome situation in *alata* in which it was found that 73 out of 79 plants possessed this number. These results are in conflict with Christoff's (1928) finding of 16 for the somatic and 8 for the gametic chromosome number. Of course the possibility exists that there may be 16-chromosomed strains of *alata*, but the present study indicates that at least in the races of *alata* used there is little chance of obtaining 8_{II} plants. It would also seem from the evidence here presented that Christoff's argument for 8 as the haploid number, on the basis of the number of univalents seen in hybrids between *alata* and species having 10 chromosomes as the haploid number, is not conclusive. Non-conjunction consistently appears to be characteristic of *alata* plants possessing extra chromosomes. This suggests that in interspecific hybrids involving this species non-conjunction may take place and would account for the finding, by Christoff and others, of P.M.C. in which 2 univalents were seen off the I-M plate and more than 2 lagging chromosomes in the homotypic division.

The non-appearance of monosomic plants in crosses of monosomic with normal is rather unexpected. Theoretically, if the unpaired chromosome of the 17-chromosomed parent divided regularly at I-M or II-M, equal numbers of gametes with 9 and 8 chromosomes should be formed, and equal numbers of 17- and 18-chromosomed plants produced in crosses with normal. If, however, the unpaired chromosome tends to lag at either division and is not included in one of the four resulting nuclei, the number of 8-chromosomed gametes should be more than 50 per cent. The fact that no 17-chromosomed plants appeared indicates that some factor or factors were operating to eliminate 8-chromosomed gametes or 17-chromosomed zygotes.

In the case of the pollen, the non-transmission of the monosomic is probably largely due to competition and differential rate of pollen tube growth. This is suggested by the fact that the monosomic plant used was obtained after controlled pollination. Low transmission of an unpaired chromosome through the pollen has before been reported. Thus in the case of the trisomic globe mutant of *Datura*, Blakeslee (1921) found the character to be transmitted to only about 2 per cent of the offspring through the pollen, and Buchholz and Blakeslee (1927) account for this by the slower growth rate of $n+1$ pollen grains as compared with normal n pollen grains. Clausen and Goodspeed (1926) found 2 per cent transmission of the monosomic "fluted" through pollen grains in *N. tabacum*. Kihara (1924) found a differential death rate eliminating pollen with extra chromosomes in *Triticum* hybrids. Lesley (1928) reported the extra chromosome to be transmitted to less than the expected proportion of progeny in the case of nine trisomic types in tomato. On the other hand, Longley (1927) found in maize plants with supernumerary chromosomes no indication that male gametes with extra chromosomes are less effective in bringing about fertilization than those with the normal chromosome number.

In the case of the female, the non-functioning of 8-chromosomed gametes is perhaps even more unusual. Thus the monosomic character "fluted" in *N. tabacum* is transmitted to about 60 per cent of the offspring through the ovules. However, in the case of maize plants with more than 20 chromosomes, Longley (*loc. cit.*) found that in the selection of an embryo-sac mother cell from the linear tetrad, there is a differential death rate in favor of female gametes with normal chromosome number. It would seem that there must have been some such differential death rate in the selection of an embryo-sac mother cell in *alata*. This would mean that with two megaspores containing 9 chromosomes and two containing 8 chromosomes one of the former would uniformly build the embryo sac irrespective of its position in the linear tetrad. No direct cytological evidence that any but the chalazal megaspore functions, has been obtained, but Palm (1922) has found that the embryo sac in *Nicotiana* may be derived from any of the four megaspores.

In this connection, it should be pointed out that the diploid megaspores produced by the monosomic were functional and produced triploid and hypotriploid plants. In the case of diploid megaspores

no differential death rate operates, for following a diploid metaphase in an E.M.C. a linear dyad would be formed and the functioning of either cell would produce a diploid embryo sac.

The occurrence of a series of hyperdiploid numbers in the progeny of the monosomic *N. alata*, suggests the situation in *Zea Mays* L. where Randolph (1928) has found a pronounced variation in the number of chromosomes in different plants to be characteristic of certain, relatively few, cultures. Although all plants with chromosome numbers deviating from the normal in maize have more rather than less than the normal complement, it would seem that the variation in chromosome number characteristic of certain cultures is probably due to the effects of an unbalanced chromosome complement in some ancestor. In *alata* the unbalance in the chromosome set of the monosomic was apparently responsible for plants with supernumerary chromosomes, and these plants in turn possess unbalanced sets which would in time no doubt give rise to cultures such as those found in maize. It is of interest in this connection to note that Randolph and McClintock (1926) found that triploidy occurs in *Zea*. It may be that maize plants with unbalanced chromosome sets tend to produce diploid gametes, as do the *N. alata* plants with unbalanced sets, and the resulting triploidy might be responsible for considerable variation in chromosome numbers, as Randolph (1928) suggests. (Cf. also McClintock, 1928).

As to the origin of variations in chromosome number, the underlying causes may be similar in *Zea Mays* and in *N. alata*. Fisk (1927) occasionally noted the early separation of the members of one bivalent in the first division, and suggested that there may be less close conjugation between the members of one or more pairs of chromosomes in certain of the sugar corn strains. Some second metaphase counts of 9-11 were noted, and Fisk suspects this to be due to the non-conjunction (asynapsis) of one pair in I-M, or possibly to non-disjunction. Although non-disjunction and non-conjunction are probably not so characteristic of *Zea Mays* as of *N. alata*, the facts that they have been observed in both species, and that, in both species, plants with deviating chromosome numbers occur, suggest that the processes of non-disjunction and non-conjunction play an important rôle in the origin of races characterized by chromosome numbers other than the number usual for the species.

In a single population of 22 plants of *alata*, evidence was obtained of both somatic and gametic chromosome doubling. Jörgensen (1928), on the basis of his experimental evidence, suggested that somatic tetraploidy occurs more frequently and is of more importance from an evolutionary point of view than is the production of diploid gametes. Two observations in the case of the *alata* plants studied, suggest, however, that this latter method of origin of tetraploid forms must not be overlooked. In the first place diploid gametes, both female and male, are produced in comparatively large numbers by certain plants. In the second place, these gametes are functional at least in the female and presumably also in the male, although diploid pollen grains may be slow in germinating. With controlled pollination or under similar conditions where the number of competing pollen grains is not greatly in excess of the number of ovules, tetraploids should occasionally be produced by the union of two diploid gametes. On the other hand, the observed polyploidy in somatic tissue is restricted to root tips as reported above and also by Lesley (1925), Langlet (1927), Hollingshead (1928), Ruttle (1928), and Webber (1929). It would therefore appear that somatic and gametic chromosome doubling are both occurring simultaneously and continuously in pure species as well as in hybrids and that neither one can be considered to play the chief rôle in the production of tetraploid species with our present limited knowledge as to the actual mode of origin of existing tetraploid forms.

Finally, it may be pointed out that *N. alata* appears to be particularly favorable material for cytogenetic studies of the method of origin, and of the transmission and chromosomal constitution of variant plants in a pure species. Further investigations of some of the problems mentioned, and the study of plants of other chromosomal constitution occurring in succeeding generations, are being carried on, and will be reported at a later date.

For material used in this study and for helpful interest and advice throughout the course of the work, I am indebted to Dr. T. H. Goodspeed, under whose direction the investigation has been carried out.

SUMMARY

1. The normal somatic chromosome number of *N. alata* is 18.
2. Differences in size, the position of constrictions, and the occurrence of satellites provide morphological distinctions which place the nine pairs of somatic chromosomes in five morphological groups.
3. From a monosomic plant obtained through controlled pollination, two populations totaling 79 plants have been grown and examined cytologically. Of 57 plants from the cross, normal ♀ × monosomic ♂, only 2 showed chromosome numbers other than 18, both plants having 19 somatic chromosomes. Of 22 plants from the cross, monosomic ♀ × normal ♂, 4 showed deviating chromosome numbers—22, 25, 26, and 27. One normal diploid plant had three tetraploid roots.
4. Of the seven plants with supernumerary chromosomes, only two, those with 22 and 25 chromosomes, showed marked distinction from normal in external morphology. A Mendelian character, pistillody of the stamens, occurred in a 3:1 ratio in the progenies studied.
5. In trisomic plants, non-conjunction of one or two pairs of chromosomes frequently occurred, resulting in II-M counts of 8-11 instead of 9-10. At II-M in a trisomic plant considerable numbers of P.M.C. contained a single large spindle with 19 chromosomes, and dyads constituted 12 per cent of the P.M.C. at the tetrad stage.
6. It is suggested that non-conjunction, non-disjunction, and diploid gametes are responsible for the deviating chromosome numbers found.

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INHERITANCE IN NICOTIANA TABACUM

IX. MUTATIONS FOLLOWING TREATMENT WITH X-RAYS AND RADIUM

BY

T. H. GOODSPEED

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INHERITANCE IN NICOTIANA TABACUM

IX. MUTATIONS FOLLOWING TREATMENT WITH X-RAYS AND RADIUM

BY

T. H. GOODSPEED

Progenies from meristematic or reproductive tissues subjected to high frequency radiation indicate an extreme lability of the hereditary constituents of *Nicotiana tabacum* and *N. rustica* (Goodspeed, 1929a, b). In contrast to the reaction of these polymorphic 48-chromosomed species, such monomorphic, 24-chromosomed species of *Nicotiana* as *glutinosa* and *sylvestris* appear to be difficult to alter by treatment with x-rays or radium.

As indicated in previous reports, extensive disruption of nuclear materials follows treatment of *tabacum* and *rustica*. Its products include the more familiar non-disjunction and fragmentation effects, and, in addition, alterations reflected in diaphase and I-M non-conjunction, and in sectional rearrangement and realignment to various and varying extent. Evidence in hand makes clear the practical difficulties in attempting an analysis of the genetic and cytological nature of a certain proportion of the variant plants which appear in such great numbers in the immediate progenies from the x-rayed tissues of these two species. A number of lines have, however, been continued from two to four generations. They are yielding significant cytological and genetic evidence as to gene alteration, the character of chromosome breakage and fragmentation, and its resultants. Moreover, stable derivative races, far removed from the control in external morphology and chromosome constitution, can readily be obtained in X_2 and X_3 and offer opportunity for detailed studies.

The present report deals with the occurrence in progenies from x-ray and radium treatments, of three less involved instances of apparent gene alteration among a far greater number which obviously are present in lines that have not attained sufficient stability in chromosomal organization or behavior to make ready analysis possible.

* The investigations in part reported upon here were made possible by grants from the Board of Research, University of California; The National Research Council (Committee on the Effects of Radiation upon Living Organisms); and through the generosity of Mr. Henry S. Chafee, of Providence, R. I., and his family.

Of the numerous varieties of *N. tabacum* under cultivation in the University of California Botanical Garden, var. *purpurea* has been grown in pure line for many years and has been largely employed for genetic analysis in inter- and intra-specific hybrids. It was the variety used in the original experiments dealing with the effects of x-ray and radium upon this species of *Nicotiana* in which occurred the genic alterations to be discussed here. It has been described and figured in earlier reports, as has the x-ray technique employed (Goodspeed, 1929a, b). In what follows, alterations in flower color and flower form and the occurrence of albino seedlings are treated of. The first two of these discontinuous changes in character expression followed x-ray treatment of sex cells. Albino seedlings appeared in seed of R_1^1 in a line derived from dry seed which had been exposed for 47 hours to 50 mg. of radium salt shielded to exclude all but gamma rays, the seeds and filter being almost in contact.

A number of other varieties of *tabacum* react as does var. *purpurea* to the effects of high frequency radiations, and in particular such races of commercial importance as Connecticut Broadleaf and Maryland Mammoth.

Acknowledgment is made to Miss Priscilla Avery for valuable assistance in the carrying on of these investigations.

FLOWER COLOR

In X_1 from x-rayed tissues, flower colors in a series representing shades of the red of *purpurea*,² the control, are characteristic of the numerous variant individuals. Shades from "rose pink" to "Bordeaux" of the Ridgway scale are found—the flower color of *purpurea* corresponding most closely to "pomegranate purple." The majority of these flower color variations were to be seen in culture 27154. This population, numbering 168 plants, came from seed produced by a flower which was x-rayed as a bud in which the pollen was rather well matured and the megaspore mother cells in early meiotic stages.

27154P₂₇ was a replica of the control in flower color and all other details of external morphology except its flower size, which was

¹ The designations "R" and "X" are used to refer to the progenies derived from the treatment of sex cells, seeds or seedlings with x-rays (X_1 , X_2 , etc.) or radium (R_1 , R_2 , etc.).

² The term red has been used in previous studies to designate the recessive flower color of *N. tabacum* var. *macrophylla*, and the term carmine for the dominant color of var. *purpurea*.

slightly reduced as to length. Its fertility was high. Since it was classified as "normal" or "normal?" no cytological material was taken from this plant. Its progeny—28335—consisted of 25 plants, three of which bore pink flowers, which in shade closely approximated "rose pink" of the Ridgway scale, while the remainder bore flowers whose color was identical with that of the control (cf. fig. 1). With the exception of one of the pink-flowered plants (P_1) the entire population was a replica of the control so far as other floral and all vege-

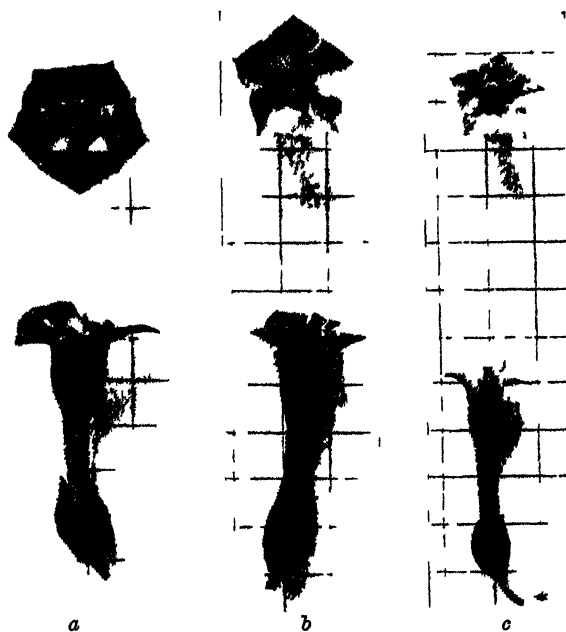


Fig. 1. Flowers: *a*, of control; *b*, of pink-flowered X_3 plant (29213 P_1); *c*, of pink-flowered haplont (29213 P_{3a}).

tative characters were concerned. From the two normal pink-flowered plants (P_4 , P_{25}) and from one red-flowered plant (P_3), progenies were grown this past summer (1929). The following table summarizes the information obtained from them.

TABLE 1
FLOWER COLOR IN X_3

Culture number	Pink	Carmine
29213 (28335 P_3 -red)	17	33
29214 (28335 P_4 -pink)	10	
29215 (28335 P_{17} -pink)	50	

As noted above, one pink-flowered plant (P_1) was variant. It differed from the control in habit, leaf shape, and flower form. It was

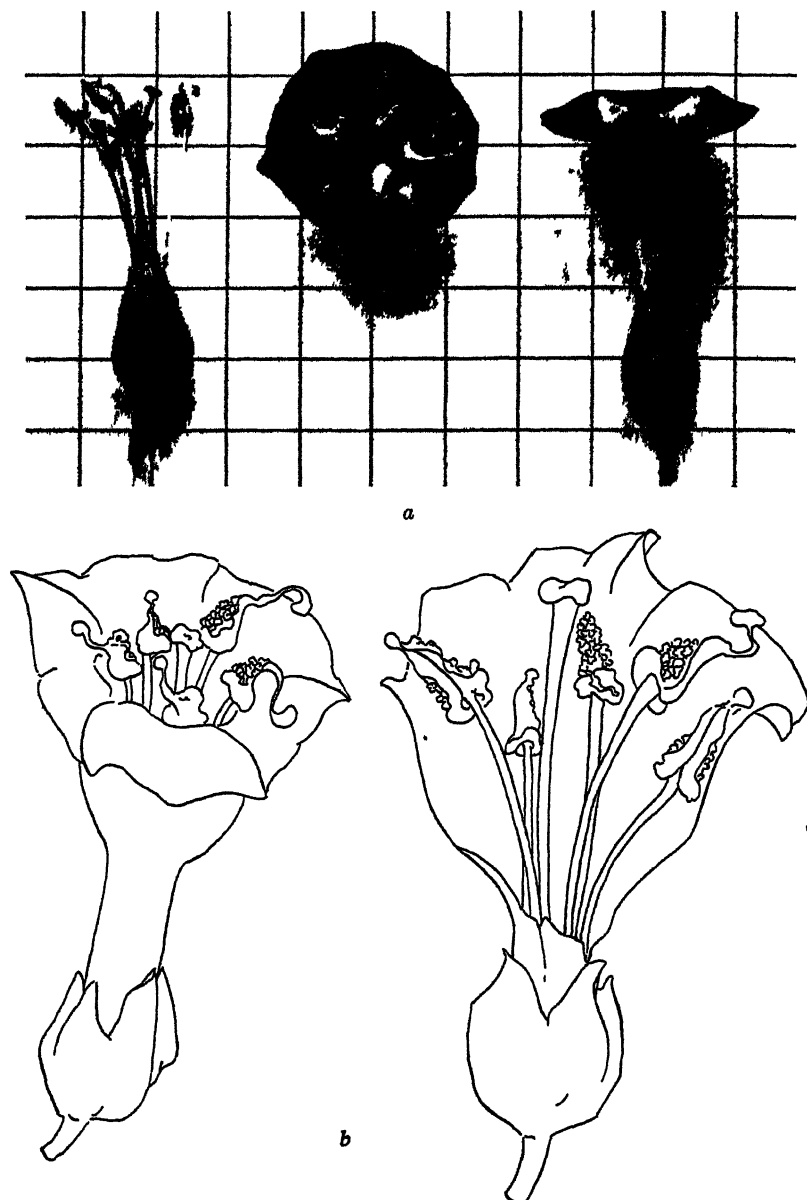


Fig. 2. a, b, flowers exhibiting pistillody of the androecium (X_a)

low and spreading rather than upright and pyramidal; its leaves were somewhat petiolate rather than sessile, with a "curly" wing and

auricle, and minor flower form abnormalities often occurred. Its flower color was, however, the same as that of the other two pink-flowered plants in the population.

The evidence in hand demonstrates the occurrence of a recessive monogenic mutation involving flower color as a product of x-radiation applied to sex cells of the tobacco plant. Inheritance of flower color contrasts in *tabacum* has been reported upon by a number of investigators (Allard, 1919; Setchell, Goodspeed, and Clausen 1922; Clausen and Goodspeed, 1922; Kelaney, 1925; Christow, 1925). Inspection and comparison in the field indicated that the pink flower color involved in this case was identical with that of certain races of this species studied here in the past and discussed in the reports referred to above. Until the completion of an analysis of its genetic relationships to these and other flower colors in *tabacum*, a discussion of the factorial constitution involved is not possible.

In one of the X_3 progenies (29213) a haplont occurred. In external morphology it was a replica on a reduced scale of sister (pink-flowered) plants. Four haplonts have appeared in x-ray progenies of *tabacum*, two in X_1 and two in X_3 . Their occurrence is of special interest because their more simplified cytological condition (cf. Ruttle, 1928; Chipman and Goodspeed, 1927) should make possible a closer analysis of such alterations in chromosome structure as they represent, especially in somatic mitoses.

PISTILLODY

Striking changes in flower form characterize many plants in the immediate progenies from treated seeds, growing points of seedlings, or sex cells of *tabacum*. Marked distinctions from the control are seen in flower size, both length and spread of corolla and length of calyx being increased or diminished to give a variety of flower size types. Similarly the shape of the limb and tube, involving lobing, fringing, absence of infundibulum, etc., is subject to variation. The insertion and length of anthers and pistil are conspicuously altered in many cases, usually in company with changes in shape and size of calyx and corolla but at times unaccompanied by such changes. Exsertion of the stigma and anthers or the reverse occurs in a number of combinations. Very conspicuous are the alterations in form and structure of the stamens including adnation, fimbriation, petalldy, and pistilldy. One instance of the last type of teratological variation is described in what follows.

In X_2 from an individual which was unaltered in any feature of external morphology, except that the flower size was somewhat reduced, plants exhibiting a marked degree of pistillody of the androecium appeared. The parent, 27154P₄₁, a sister plant of the one in which occurred the flower color mutation just described, was not examined cytologically. In its selfed progeny (28338) four plants showed the type of anther abnormality illustrated in figures 2a, b. The presence of a small, normally formed style and stigma was the most conspicuous feature of this teratological variation.

A somewhat similar condition of the androecium has been described in *Nicotiana alata* by Costerus (1907) and has arisen on two different occasions in cultures of this species in the University of California Botanical Garden. White (1913) has discussed the occurrence and inheritance of a corresponding teratological expression which appeared in a segregant of *N. Langsdorffii* \times *N. alata*. Apparently pistillody has not previously been described in *tabacum* and in general it is a rather rare type of teratological variation.

At the start of the flowering season practically no viable pollen is produced in flowers of this type, whereas towards its close the anthers uniformly exhibit less structural abnormality and contain a considerable proportion of viable pollen. The following table details the inheritance of pistillody in the progenies investigated. It should be noted that pistillody was always fully expressed and not variable in expression from plant to plant, from flower to flower, or among the anthers of an individual flower.

TABLE 2

Culture number	Pistilloid	Normal
28338	4	45
(27154P ₄₁ -normal)		
28571*	50	----
(28338P ₄₁ -pistilloid)		
29220	23	----
(28338P ₄₁ -pistilloid)		
29218	25	----
(28338P ₁₄ -pistilloid)		
29217	4	45
(28338P ₁ -normal)		
29221	----	24
(28014P ₂₁ \times 28338P ₄₁ - control \times pistilloid)		

* Grown in the greenhouse, winter, 1928-29.

Among the X_2 plants (28338) which showed no evidence of pistillody there was considerable variation in flower size. In some 5 plants

the flower was somewhat reduced both in length and in spread and in one plant a significant enlargement in flower size was exhibited. From one small-flowered plant, 29217 was grown. As will be noted in table 2, the number of plants possessing pistilloid anthers was the same as in X_2 (28338). As in this latter population, there were, in 29217, plants bearing smaller flowers. The reduction in flower size was significant in the case of 5 or 6 individuals.

Cytological examination was made of a number of X_3 individuals which exhibited pistillody. As already indicated, the anthers were highly abnormal and relatively very little viable pollen was produced by them. It was possible, however, to obtain aceto-carmin smears and also paraffin sections in which chromosome number and behavior in P.M.C. could be studied. It is proposed to describe elsewhere the histological and cytological peculiarities which occur in these anthers. It may be noted, however, that the X_3 chromosome garnitures gave no visible evidence of alteration.

The data submitted indicate that in this case, also, a recessive gene alteration was produced by the x-ray dosage employed. Although the number of individuals in the X_1 and X_3 progenies is not large, the situation involved is demonstrated by the type of inheritance which they show. White (*loc. cit.*) found that petaloidy was completely recessive in two F_1 families, the F_2 giving a close approximation to a simple 3:1 ratio. In "what appears to be another hybrid F_1 family" it was wholly dominant. Certain other teratological characters in *tabacum*, such as fasciation, exhibit complete to partial dominance and behave as a single genetic factor or are conditioned by a considerable number of factors.

CHLOROPHYLL DEFICIENCY

A full description of the effects upon the immediate progeny of treatment of seeds and sex cells of *tabacum* with the gamma rays of radium is in preparation. Here it may be said that there appears to be a rather limited dosage range which is effective in the case of dry seeds. Below this range there is little evidence of the production of quantitative or qualitative alterations in nuclear materials and above it the effect is highly lethal. Within it there is very little reduction in viability and approximately 25 per cent of R_1 plants are variant. In one such population (28518) albino seedlings appeared when the seed of one R_1 plant was germinated. There were some 20 plants in the R_1 culture and 4 of them showed distinctions in external mor-

phology as compared with the control. Of these four, P_{17} was the most outstanding by reason of its increased flower size. The stigma was, also, somewhat unusually exerted and the fertility a little reduced.

The seed of 28518 P_{17} gave heavy germination and approximately 25 per cent of the seedlings were uniformly a light shade of yellow, soon fading to chalky white, and they failed in an early cotyledon stage without production of any chlorophyll. From the green seedlings a population of 44 plants (29275) was grown. In this culture there was sharp segregation as to flower size and flower color. There were 12 plants with flowers that were distinctly larger and in which the tube was almost white within and without, to 32 plants which were replicas of the control both in flower size and the colored condition of the corolla tube. The flower color contrast was very striking and was referred to in classification as "white throat."

Seed of 9 R_2 plants was germinated under controlled conditions and table 3 details the occurrence of albino seedlings in these progenies.

TABLE 3
OCCURRENCE OF ALBINO SEEDLINGS IN R_2

Seed of 29275	Seedling color		Total germination
	green	yellow	
P_2	76	22	98
P_3^*	58	16	74
P_{13}^*	53	17	70
P_{19}	74	23	97
P_{23}^*	64	19	83
P_{21}	69	29	98
P_{34}^\dagger	97	97
P_{40}	75	23	98
P_{48}	73	27	100
Totals	542	176	718
Calc.			
3:1	539	179	718

* These plants were of the "white throat" type. \dagger Omitted in totals.

The "white throat" condition is presumably a product of quantitatively altered chromosomal relations. At I-M in P.M.C. there were $22_{II} + 1_{III} + 1_I$, and anomalous chromosome behavior was frequent, giving a considerable amount of chromosome elimination through lagging at I and II. Sister plants, which were replicas of the control in external morphology, were equivalent to the control in cytological features also. The lowered viability of seed of plants of the "white throat" type (cf. table 3) presumably is not to be referred solely to the unbalanced chromosomal condition they exhibit. At any rate,



a



b

Fig 3 Green and albino seedlings in R_2
(a) 29275P₂₂, (b) P₂ (cf table 3)

"normal" sister plants, in a few instances, gave a much reduced set of seed on selfing. The apparent occurrence of additional types of lethality in this line is being investigated. Seed of $P_{3,4}$ gave all green seedlings as shown in figure 3, where germination in P_2 (cf. table 3) is also illustrated.

It seems clear that a recessive gene alteration has again followed treatment with high frequency radiation; involving in this instance the occurrence of a lethal factor. Although chlorophyll-deficient races are well known among the cereals and in other plants they have not before been reported in tobacco. Strains in *Nicotiana tomentosa* with white-margined leaves occur and they, of course, produce only chlorophyll-deficient seedlings which die in an early cotyledon stage. In the past we have had records of the appearance of albino seedlings in our cultures of two or three other *Nicotiana* species but no information was obtained as to the inheritance of this condition. Stadler (1928) obtained chlorophyll deficiencies in barley as a result of x-ray and radium treatment.

As was to be anticipated from the predominant occurrence of lethal mutations in corresponding types of experiments in *Drosophila*, lethality has continually been evidenced in the various progenies of *tabacum* grown from tissues subjected to high frequency radiation. Under the morphological and physiological conditions obtaining in the higher plants only such "visible" zygotic conditions of lethality as involve viability of seed and chlorophyll deficiencies can readily be investigated. Out of a larger number, two cases in which histologically normal seed exhibits a high degree of non-viability are being studied. A doubtfully successful attempt was made to determine the percentage of failures to set seed in the case of an individual whose pollen gave full seed capsules on the control but defective capsules upon selfing.

DISCUSSION

In common with other economically valuable crop plants, tobacco has been the subject of many investigations of a quasi-genetic character. In particular a number of efforts have been made to classify the more representative varieties of this highly polymorphic species. Based largely upon morphological criteria, they have accomplished little more than a further demonstration of the extent to which genic modification has been occurring over the considerable period that this species has been in cultivation. In contrast to the high degree of variability exhibited by the present-day assemblage called *Nicotiana*

tabacum, the occurrence of what may pass as gene mutations reported in controlled experiments is rare. The situation in this connection has been summarized by East (1928, p. 294).

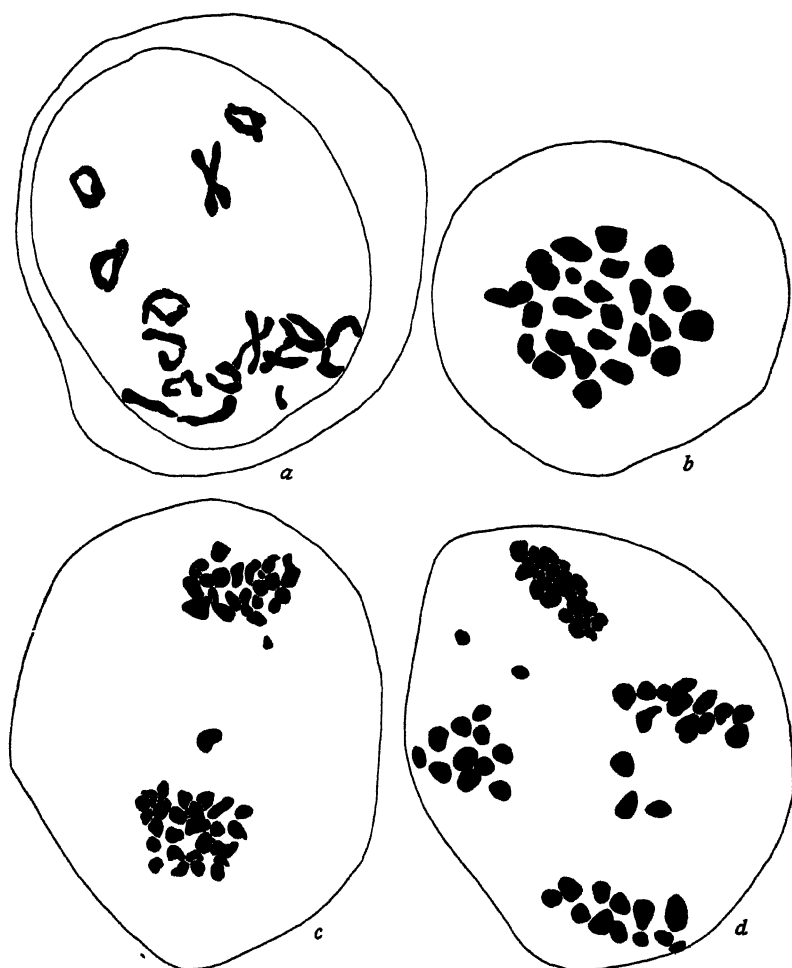


Fig. 4. P.M.C. of pink-flowered variant (28335P₁): a, diaphase showing imperfection of conjugation in the case of two pairs and the occurrence of a chromosome fragment, b, I-M, 24_H plus 1 fragment, loose conjugation of one pair; c, II-M, fragment "divided" and one chromosome lagging; d, early II-T, showing chromosome lagging.

On this ground alone the occurrence of the discontinuous variations following treatment with high frequency radiation which are reported upon above might be considered to be significant and not of spontaneous origin. In addition, the information gained from cytological studies of chromosome constitution and behavior in the

progenies involved gives evidence that these new character contrasts are intimately related to the effects of the treatment employed.

For example, in the progenies exhibiting the discontinuous flower color variation, P_1 (28335), as already noted, differed from the control in certain features of external morphology in addition to flower color. The character of its chromosome complement and of chromosome behavior at meiosis is, in part, illustrated in figure 4. There were apparently 24_{II} plus a rather large fragment, and loose conjugation of at least two of the chromosome pairs was observed from diaphase through I-A. As a result of this latter situation counts at I-M of 26 units, of which one was clearly a fragment, were not infrequent. The fragment was always seen at diaphase and was usually included within the equatorial plate of bivalents at I-M. It divided in some cases at I-A as indicated by II-M counts of 25 units, of which one was a smaller fragment. Ordinarily the fragment, or the product of its division, was included in the interkinetic nuclei, but, rarely, it remained in the plasma as shown at I-T and II-M. Chromosome behavior was, in general, quite normal during I, but during II was often irregular. Lagging was frequent and involved products of I-A distribution in addition to the fragment or its "halves."

In the case of P_4 which was pink flowered but otherwise equivalent to the control in external morphology, a cytological situation similar to the one just described for P_1 was indicated. In the case of P_{25} , the remaining pink-flowered plant, the counts at I and II-M gave 24_{II} of normal size. They disjoined and were distributed in conventional fashion. The fertility of both P_1 and P_4 was low, indeed P_1 gave almost no selfed seed, while that of P_{25} was normal. Correspondingly, it was possible to bring to maturity a progeny of only 10 plants in the case of P_4 whereas a full population of 50 plants was grown from seed of P_{25} and also from P_{27} , the latter being a normal, red-flowered, fully fertile plant which was not examined cytologically. Cytological examination of the X_1 progenies showed a chromosome complement equivalent to that of the control and its conventional behavior during the meiotic divisions.

The combined evidence relates intimately the flower color mutation to the effects of the x-radiation originally applied. Apparently the presence or absence of the products of the chromosome fragmentation observed is not concerned in the inheritance of the flower color change. Indeed, it appears that the undoubted presence of chromosome fragments, as in $27154P_{27}$ or $28335P_4$, need not be reflected in major

changes in external morphology, a point previously noted in another connection (Goodspeed, 1929b). Possibly in some such cases the fragment or fragments present represent the essential constituents of disorganized or otherwise obliterated chromosomes or simply detached portions of the chromosomes the remainders of which are themselves present also. However this may be, there is here evidence of quantitative chromosomal alteration accompanied by, but independent of, a recessive gene alteration affecting flower color which in X_2 and X_3 exhibits segregation according to Mendelian expectation.

As contrasted with the employment of stocks in which the effects of x-rays and radium upon specific factor contrasts could be investigated, the particular series of experiments reported upon above was not so appropriate for demonstration of the occurrence of gene mutation, especially as only a beginning has been made at genetic analysis of the many allelomorphic contrasts exhibited in intervarietal hybrids of *Nicotiana tabacum*. Begun simultaneously with and independently of the work of Muller (1927), our original experiments immediately demonstrated the extremely responsive nature of all meristematic and sporogenous tissue of the tobacco plant to x-radiation and led to similar investigation in the case of other species of *Nicotiana*. Particular emphasis has been placed upon occurrence of significant cytological consequences and it was early found that clearly visible chromosomal alterations of the most basic sort reappeared in later generations. There is good reason to suppose that such fundamental and viable quantitative and structural alterations in the hereditary material would not originate in the absence of correspondingly fundamental qualitative changes. The data present here indicate that the inheritance of the induced character expressions described is in accordance with Mendelian expectation in the case of a monogenic mutation. It should, however, be noted that the occurrence of Mendelian ratios may not be a demonstration of actual monogenic change since any type of alteration in a chromosome which does not reduce the viability of the gametes which receive it and which does not affect its conjugation with its unaltered homolog may be reflected in typical Mendelian ratios. Thus, in recent investigations designed to indicate the genetic effects of high frequency radiation it is, perhaps, a question as to how accurately, in the present state of our knowledge, the extent of genetic mutation reported can be ascribed exclusively to qualitative as contrasted with quantitative effects upon chromosome content and configuration.

SUMMARY

Three recessive monogenic mutations induced by treatment of *Nicotiana tabacum* var. *purpurea* with x-rays and radium are described. They involve pink flower color, pistillody of the androecium, and albino seedlings.

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OCCURRENCE OF TRIPLOID AND TETRAPLOID
INDIVIDUALS IN X-RAY PROGENIES
OF NICOTIANA TABACUM

BY

T. H. GOODSPEED

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BY

T. H. GOODSPEED

Cytological investigation has demonstrated the occurrence in nature of polyploid individuals in a number of plant species, but no such forms in *Nicotiana tabacum* have been reported. Considerable importance attaches to this species by reason of its cytogenetic relationships to other species of the genus and its cytology has been under investigation here and elsewhere (cf. Chipman and Goodspeed, 1927; Ruttle, 1928; Rybin, 1927). It is thus of some interest that, among an assemblage of plants representing products of treatment with x-rays, one triploid, *N. tabacum* var. *purpurea*, and one tetraploid, var. "Maryland Mammoth," have been found. They apparently represent products of processes which, under natural conditions, may often be responsible for the spontaneous occurrence of polyploid plants.

TRIPLOID, var. *purpurea*

This plant, 28473P₁₁, was of the following derivation. An X₁ progeny, 27G110, was grown from selfed seed produced by a flower which as a bud was treated for 20 minutes with x-rays. The treatment involved 75 kv., 2.8 ma., at a target distance of 30 cm., no filter being used. In this bud the pollen was partly matured and E.M.C. were about to be differentiated. Plant 5 of 27G110 was distinctly different in external morphology from sister, untreated plants (control) in that it possessed shorter internodes; slender, petiolate leaves rather than broad, sessile ones; and a flower in which the stigma was markedly exserted. The flowers of this plant also showed distinctions from those of the control in shape and size of corolla and calyx. Its pollen was highly defective and no selfed seed was matured. From 27G110P₃ ♀ × control ♂, seed was obtained, however, and from it an F₁, 28473, consisting of 28 plants, was grown.

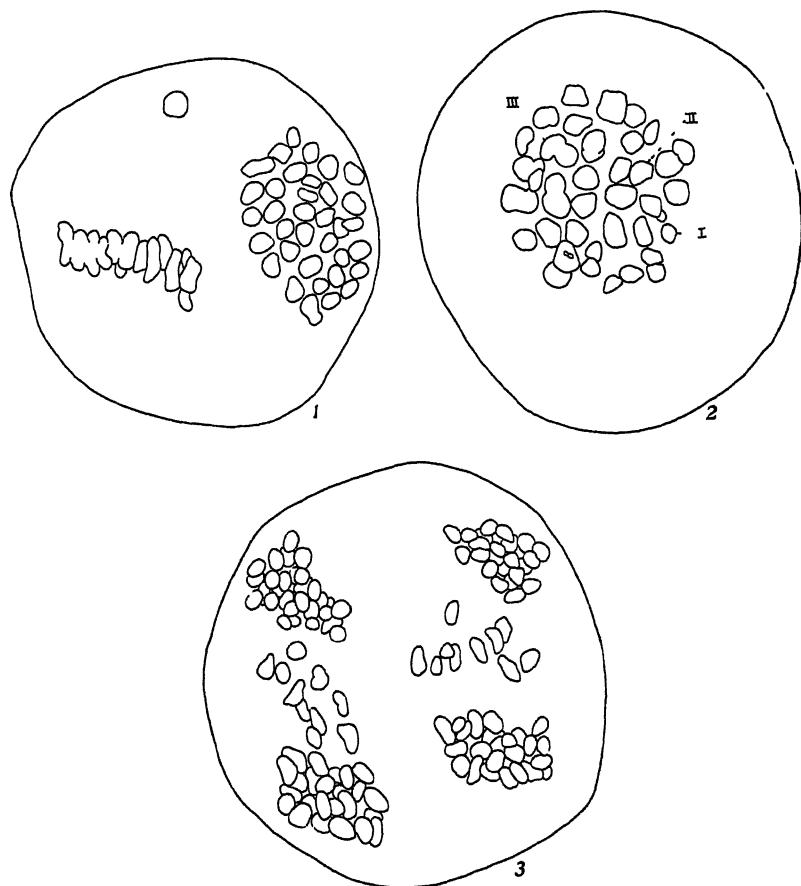
Considerable variation in the expression of certain plant characters, and particularly those of the flower, was shown in 28473. In the control the stigma is found to be just below the top of the corolla tube and the anthers slightly higher. Late in the flowering season the anthers may be somewhat exerted but, earlier, any appreciable distinction in length of filaments or style is significant and when extreme is not difficult to identify in classification of a population of *tabacum*. In 28473 there were 8 plants with both anthers and stigma exerted, 8 with anthers exerted but stigma in normal position, 1 with anthers exerted and stigma low in the tube, and 1 plant with anthers normal and stigma low. Four plants showed more or less strikingly petiolate leaves. In addition there were in this population, 10 plants which were classified as equivalent to control in external morphology.

The eight plants which showed both anthers and stigma exerted formed a definite "type." All were of low habit, with stiff, twisted, and abnormally shaped leaves and lowered fertility. One of these plants (P_{11}) was examined cytologically and proved to be a triploid. On the basis of equivalence in external morphology, it is probable that all eight were of this chromosomal constitution. In the other classes of variant expression included in 28473, three plants were examined cytologically. Two of them were trisomics and showed $24_{II} + 1_I$ at I-M (*tabacum*— $n = 24$) and the other was a monosomic with $23_{II} + 1_I$.

No cytological information is available as to the constitution of the original x-ray variant (27G110P_g) which was a parent of 28473. The fact that its pollen was highly defective suggests that it was a product of induced quantitative chromosomal alteration. In external morphology it somewhat resembled the triploid group in 28473 but in view of the large number of plants in this group (8 in a population of 28) it is not reasonable to suppose that it was itself triploid. Presumably, it was chromosomally unbalanced and, like so many X_1 variants (cf. Goodspeed, 1929), possessed one or two chromosomes in addition to the usual diploid set.

Quantitative chromosome unbalance has been shown to induce meiotic aberrations of various sorts. Thus, the trisomic condition may be accompanied by evidence of non-conjunction and may give rise to the production of somatic gametes in a frequency far higher than is otherwise observed (cf. Avery, 1929). It is therefore suggested that 27G110P_g was an induced trisomic, which, because of its unbalanced chromosomal condition, produced considerable numbers

of somatic gametes and also certain $n-1$ gametes as a result of non-conjunction, as well as $n+1$ and n gametes. When crossed with control male, triploid, monosomic, trisomic, and presumably other chromosome types were produced in 28473.



Triploid *N. tabacum* var. *purpurea* in an \bar{X}_1 progeny

Fig. 1. II-M, 35 + 1 in plasma.

Fig. 2. I-M, approximately $12_{III} + 12_{II} + 12_I$. One unit belonging to each category is appropriately designated.

Fig. 3. II-T, showing ten laggards in each spindle.

The exact number of chromosomes in the triploid *tabacum* plant (28473P₁₁) could not be determined with entire certainty from the counts made at I and II-M. That the number was close to, if not identical with, a full triploid complement is shown by the fact that the most frequent count in single II-M plates was 36 (fig. 1). At

I-M the total number of units varied from 36 to 42 and in all cases it was clear that univalents, bivalents, and trivalents were present. When, as often happened, the total number of units at I-M was 36 they could with close to certainty be classified as $12_{III} + 12_{II} + 12_I$ and this is taken to be the typical type of I-M configuration (cf. fig. 2). Frequently the total number was greater than 36 with a consequent increase in number of univalents and bivalents and decrease in number of trivalents. Thus in a I-M plate totaling 37 there were $11_{III} + 13_{II} + 13_I$ and in one with 39 units there were $9_{III} + 15_{II} + 15_I$. Often the conjugation of the three members of a trivalent was not equally close, with the result that its interpretation as a trivalent or as a bivalent and a univalent was purely a matter of individual opinion.

As indicated by the infrequent occurrence of chromosomes in the plasma at II-M (cf. fig. 1), the first meiotic division was comparatively regular. Univalent division was relatively rare although in certain P.M.C. unusually small chromosomes, often at a level different from that of the II-M plate, were taken to be products of such division at I-A.

By contrast with the relatively regular meiotic behavior during I, in this triploid plant, a large amount of chromosome lagging appeared at II-A in practically every P.M.C. examined. The lagging chromosomes were scattered from one pole to the other or were oriented in a rather definite zone midway between the II-T chromosome groups. At early II-A the size and behavior of these chromosomes suggested that they represented bivalents undergoing disjunction. Further, the range of their number closely approximated that of the trivalents observed at I-M. It is possible that the lagging chromosomes at II-A represent products of non-disjunction of two of the three constituents of the original trivalents. They might disjoin in early II-A, while the products of normal disjunction during I are dividing, and themselves divide at early II-T, this late division being perhaps reflected in the much scattered appearance of II stages, already referred to (cf. fig. 3).

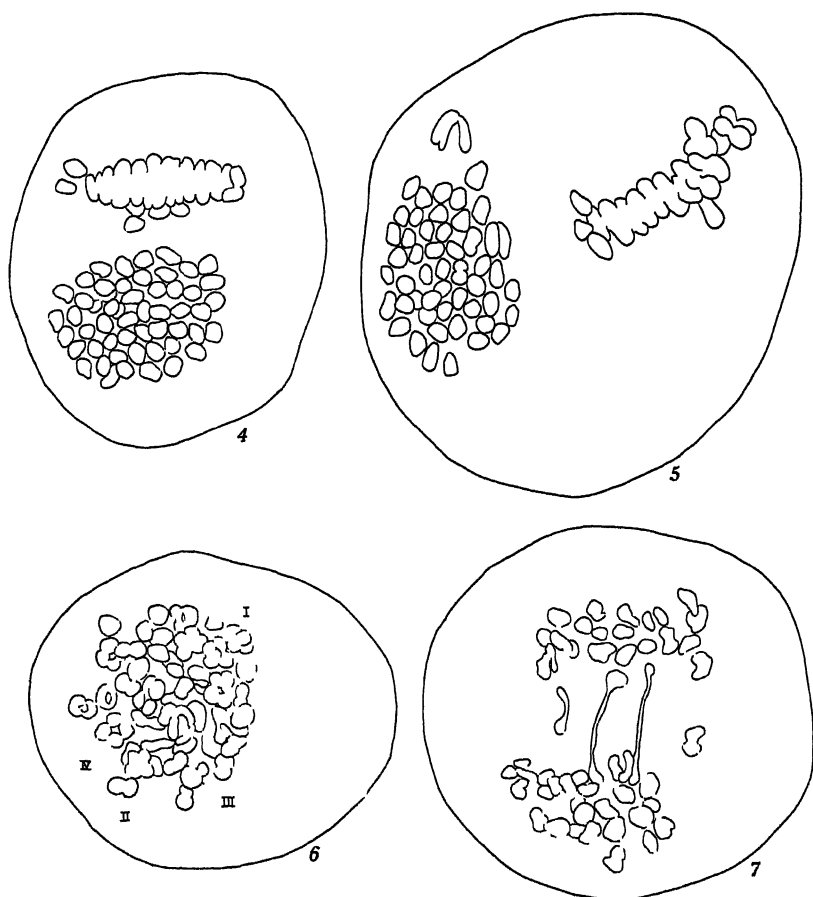
TETRAPLOID, var. "Maryland Mammoth"

In the spring of 1928, seed of the commercial variety of *N. tabacum* called "Maryland Mammoth" was treated with x-rays and radium and in some instances treatment was given to young seedlings with and without previous treatment as seeds. A number of progenies following such treatment were grown along with untreated sister plants. They matured very slowly and came to flower too late in the fall to permit thorough classification. Distinctions from the control in many features of the external morphology were, however, striking. A number of plants from treated seeds or seedlings were much reduced in size and vigor, which was in decided contrast to the superlatively vigorous character of this *tabacum* variety. One such small, weak plant was held in the greenhouse and it matured very slowly during its first year. It occurred in a progeny produced from seeds and seedlings which were x-rayed. With 50 kv. and 5 ma. at a target distance of 30 cm., the seed was x-rayed for 10 minutes, and 25 days after germination the seedlings were similarly treated for 45 minutes.

This plant, 28482P₅₁, finally succeeded in maturing a single, small inflorescence on a plant less than two feet high. The flowers were small and somewhat misshapen and set no selfed seed. Cut back during August, 1929, an extremely vigorous shoot soon developed from an adventitious bud just above the surface of the soil. This shoot produced numerous laterals which had a partly fasciated appearance and bore leaves which showed various evidences of tissue distortion and inversion. Large, often misshapen, flowers were freely produced but only a small amount of viable seed has been obtained from them. Examination of P.M.C. showed that this shoot possessed approximately 96 chromosomes and is thus tetraploid in character.

It would appear that the rather heavy x-ray dosage applied to the growing point of this plant in the young seedling stage was productive of suspended mitoses or failures of cytokinesis which resulted in areas of tetraploid tissue. Such tissues would presumably suffer in competition with surrounding basically diploid tissue and would early be left behind. They, then, may have become incorporated in the production of an adventitious bud which proliferated under the conditions which obtained in this case. It might be noted in this

connection that, in a number of other products of x-rayed growing points, in other varieties of *tabacum*, the presence of a mosaic of nuclear elements is indicated by the appearance of differences in



Tetraploid *N. tabacum* var. "Maryland Mammoth" in an X₁ progeny

Fig. 4. II-M, plate in polar view with 50 chromosomes, three of which are dividing.

Fig. 5. II-M, with 50 units, two of which appear to be bivalents which have not as yet disjoined, probably products of the disjunction of quadrivalents in I.

Fig. 6. I-M, approximately 38 units interpreted as $11_{IV} + 4_{III} + 19_{II} + 4_I$. One unit belonging to each category is appropriately designated.

Fig. 7. I-T, at least four units lagging and apparently undergoing division.

external morphology on the various lateral shoots of a single individual. In one case a shoot, almost equivalent to the control in external morphology, was produced from a basal bud of a highly abnormal plant in a population obtained through x-raying of imma-

ture seeds. Whatever its method of origin it seems clear that not only was this shoot of a very different chromosomal constitution from that of the original plant, but also, to judge by the somewhat altered character expression it exhibited, that its chromosome garniture contains certain of the induced alterations which were reflected in the variant nature of the original plant from which it arose vegetatively.

Although the relatively large number of chromosomes involved in this case made their accurate determination difficult, the combined evidence from increase in cell volume as contrasted with diploid, from meiotic chromosome configuration and behavior, and from such counts as were made, left no room for doubt that a tetraploid condition was involved. The most reliable evidence as to total chromosome number came from studies at II-M. The II-M plates were quite regular in appearance, with only rarely any indication of lagging during I, and the number of chromosomes in single plates varied between 47 and 51 (figs. 4 and 5).

At I-M the presence of quadrivalents of various configurations was striking, although bivalents were the most frequent in occurrence and a few trivalents and univalents appeared. One typical I-M contained 38 units of the following constitution, $11_{IV} + 4_{III} + 19_{II} + 4_I$ (fig. 6). To some extent this classification is arbitrary and the interpretation of the number of quadrivalents *vs.* bivalents was in one or two cases difficult so that the number in one class or the other may have been too low or too high. A total of from 96 to 98 chromosomes could, however, be accounted for in the I-M plates.

During I-A, disjunction and distribution were more regular than might have been anticipated. In the case of the quadrivalents it was, however, clear that complete disjunction in all instances was not consummated in that, occasionally, the products were bivalents which persisted until II-M (cf. fig. 5). In addition, a few univalents sometimes lagged and divided at late I-A (cf. fig. 7). Their number corresponded closely to that of the univalents observed at I-M. At II-M these I-A conditions were reflected in the occurrence of disjoining bivalents, halves of univalents, and chromosomes slightly off the plate. Thus, for example, in figure 5 it is clear that two of the units represent bivalent chromosomes in the process of disjunction.

A striking deviation from the typical meiotic behavior just described not infrequently occurred. Here a massive spindle appeared and upon it the entire 96 chromosomes became oriented and ultimately

divided. The products of such metaphases gave P.M.C. filled with a cloud of chromosomes of uniform size and smaller than univalents. Whether the origin of such giant spindles involved the production of restitution nuclei or fusion of II-M achromatic figures could not be determined. Their occurrence in an already tetraploid individual, leading to the possible formation of somatic gametes, is of some significance in that individuals with higher chromosome multiples might be a product of an initial incidence of polyploidy. It may be, however, that it is only in cases like this one where presumably genic unbalance has occurred or been induced that the formation of somatic gametes might occur (cf. Goodspeed and Avery, 1930).

DISCUSSION

It would appear that some significance might attach to the fact that, at I-M in both the triploid and the tetraploid individuals described above, the minimum number of units was 36 and that the maximum number of multivalents was approximately 12. In this latter connection there is a suggestion that a greater capacity for conjugation exists between the members of a certain set of 12 chromosomes which has been duplicated more than once, than between the remaining chromosomes of the triploid or tetraploid garniture. There is, however, no apparent reason why this should be the case even on the hypothesis that in the origin of *tabacum* amphidiploidy has been involved (cf. Goodspeed and Clausen, 1928).

It seems more probable that more or less mechanical factors may be concerned in the type of chromosome conjugation exhibited in P.M.C. of these polyploid individuals. Thus, the formation of multivalents may be a random affair depending upon the orientation of the homologous chromosomes and their space relations in, perhaps, the last archesporial telophases. On this assumption 12 would represent a mid-number in terms of the 24 trivalents or quadrivalent units which, theoretically, should be produced at I-M in the triploid and tetraploid. If such were the underlying basis for the observed conditions the various chromosomes involved in trivalent or quadrivalent formation would differ from one P.M.C. to the next, though the total number of multivalents were the same.

A more acceptable explanation might be furnished by a consideration of the relation of chromosome size to the extent and character

of the resulting conjugation. Darlington (1929) has suggested that chromosome conjugation at I-M is a product of chiasma formation the extent of which, in turn, is related to the length of the chromosomes involved. Thus, chromosomes whose length exceeds a certain value might be able to unite as multivalents while those below this value would be unable to attain such valency. Now, Ruttle (1928) has shown that in the somatic chromosome complement of *N. tabacum* haplonts there are at least 7 long chromosomes, 2 short ones, and 15 of intermediate length, some of which "are doubtless longer than they appear." From the point of view just referred to, it may therefore be that the seven long chromosomes when multiplied are the most constant in their capacity to form multivalents while those of intermediate length are variable in this regard, there being at least five whose length is sufficiently great to permit multivalent formation.

It has already been shown that, following treatment of *Nicotiana tabacum* with high frequency radiations, transgenations occur and that stable derivatives whose altered phenotypes depend upon induced chromosomal reorganization can readily be obtained. That polyploidy may be a by-product of induced chromosome or gene unbalance is indicated by the data presented in this report. Elsewhere further description is being given of the extreme character of the alterations in mitotic and meiotic behavior to be observed in products of treatment with x-rays or radium; of the fact that haploidy may be induced; and that even apomixis may become characteristic of derivative lines. It would therefore appear that, in the case of *N. tabacum* at least, there is almost no limit to the capacity of the hereditary material to function after induced qualitative and quantitative alterations. That they may now be induced at will in this material is making possible a variety of cytogenetic studies bearing upon fundamental problems. At the present time they point to the previously less appreciated importance, for interpretations of problems of descent, of quantitative nuclear alterations which either directly or indirectly provide viable material upon which recognized evolutionary processes may operate.

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BY

T. H. GOODSPEED

The establishment of the chromosome theory of heredity leaves no room for doubt that the invisible genes, some products of whose interactions are reflected in the features of the external morphology which characterize the organism, are contained in the visible chromosomes. Thus, it has been shown that observable alterations in the nature of a chromosome garniture or in its meiotic behavior may occur simultaneously with the origin of distinctions in external morphology. Some attention has therefore been given in modern cytogenetic investigations to the manner and extent of such alterations in chromosome complements and mechanisms, particularly in connection with the nature of those external agencies under whose influence they may be taken to occur. At the present time the most effective of such known agencies are certain high frequency radiations which are also productive of qualitative alteration in the hereditary material. Although, obviously, the full genetic significance of visible chromosomal alterations cannot be determined by direct observation, it is nevertheless valuable to enumerate such observations; for the extent to which chromosome complements can be altered without, in consequence, being eliminated, is a matter of some importance from the evolutionary point of view, and one which is susceptible of experimental determination.

In previous reports dealing with the effects of x-rays and radium upon *Nicotiana tabacum* (Goodspeed, 1929; Goodspeed and Avery, 1930) emphasis was placed upon the cytological manifestation. Earlier studies demonstrated the occurrence of certain types of alteration in

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chromosome garnitures and meiotic behavior of this species. More recently, in part as a result of detailed cytological examination of derivatives in X_2 and X_3 , the occurrence of additional categories of chromosome alteration has been better appreciated, and on the basis of this larger experience a more accurate and significant classification of the cytological manifestations in X_1 is now possible. In addition, evidence from progenies derived from treatment of embryos, seeds, and growing points as well as sex cells is now available. The present report brings together these various data.

It has long been known that irradiation during the period of greatest observable nuclear activity is followed by the most serious disruption of the protoplast. Thus in *tabacum*, treatment with x-rays of flower buds in which P.M.C. or E.M.C. are in meiosis may produce chromosome alterations on the border line of lethality or over it. Practically speaking, treatment of E.M.C. is most effective since, apparently, a greater non-viability of altered gamete sets eliminates a larger proportion of chromosome alterations produced in P.M.C. than in E.M.C. The possible extent of such alterations forty-eight hours after x-raying is shown in figure 1a. Here few chromosomes appear to be "normal" in size and shape, and these have been distributed in a characteristic manner to the two poles at late I-A. The majority, on the other hand, either lag on the spindle, or they are being irregularly disjoined or divided, or have become fragmented. Such a fundamental disruption of a chromosome garniture and its meiotic mechanism seldom, if ever, is followed by viability of the resulting gametes. A somewhat less severe disruption, however, gives a considerable proportion of viable gametes of types which, apparently, would not survive in certain other organisms similarly studied because, perhaps, they do not, as does *tabacum*, possess a high chromosome number or owe their origin to amphidiploidy.

TABLE 1

DISTRIBUTION OF TYPES OF ALTERATION IN THE TYPICAL CHROMOSOME COMPLEMENT OF *Nicotiana tabacum* EXHIBITED AT MEIOSIS OF X_1 PLANTS

Types of Chromosome Alteration

Year	Fragmentation	"Addition"	Non-conjunction	Unpaired chromosomes	Totals
1927	4	3	8	10	25
1928	----	2	3	9	14
1929	9	8	9	5	31
Totals	13	13	20	24	70

In table 1 is included the evidence in hand as to the occurrence of alterations in chromosome garnitures exhibited during meiosis of X_1 individuals. The number of different plants dealt with was 49 and since many of them gave evidence of more than one type of alteration, the total number of alterations observed was 70. A considerably larger number of plants has been examined cytologically. Some gave no characteristic indication of chromosome alteration and in others a final decision could not be made as to the precise character of the alterations involved. Since all the plants which were studied in the present connection exhibited marked distinctions from the control in external morphology, the enumeration in table 1 appears to express rather definitely the character of the altered chromosome complements of which those distinctions are a reflection.

CHROMOSOME FRAGMENTATION

Of the various types of chromosome alteration included in table 1, chromosome fragmentation was the most striking and usually the most readily identified. Throughout meiosis chromosome units appear which are smaller than the products of bivalent disjunction in *tabacum*, and which are outstanding by reason of their diminutive size and their orientation, particularly on the I-M plate. They occur most often in X_1 plants which are products of x-rayed sex cells but also, in a few cases, in individuals x-rayed during the early divisions of the zygote from which they arose.

In the former instance fragmentation leads to a variety of possibilities as to the composition of the X_1 chromosome complement in which the fragments occur. That such a complement will tend to approach that characteristic of *tabacum* is obvious, in that inviability and unfavorable competition are known to eliminate too greatly altered gametes. The fragments are characteristic of plants whose I-M plates show 23_{II} or 24_{II} . The presence of only 23_{II} suggests that the chromosome which gave rise to the fragment or fragments is not completely represented in the particular X_1 garniture. The presence of 24_{II} indicates that one of the gamete complements which produced them contained a quantitatively unaltered chromosome set plus a fragment or fragments of other chromosomes which were fragmented prior to the I-A distributions of which they are the product.

In some cases the fragments apparently represent segments of chromosomes which themselves are present at I-M either as bivalent

partners or as univalent chromosomes. This situation is particularly characteristic of X_1 plants whose early zygote divisions were subjected to x-radiation. Again, in plants of such X_1 progenies the total number of entire chromosomes present at I-M as bivalents or univalents often does not equal the typical somatic complement of 48. The number of fragments may, however, be considerable, and often there is evidence of "additions" to entire chromosomes. Thus, even in such plants a complete or almost complete somatic garniture may be present.

In volume the chromosome fragments vary from those on the limits of visibility to those which are almost indistinguishable from the smallest univalent characteristic of *tabacum* (cf. fig. 1b). Most com-

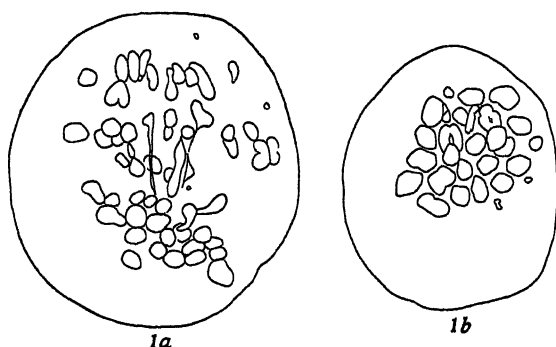


Fig. 1. *Nicotiana tabacum* var. *purpurea*. P.M.C.: a, I-M, condition 48 hours after x-raying; showing bivalent disjunction of normal appearance, fragments, lagging chromosomes, non-conjunction and division of univalents and fragments. b, 29313P, X_1 plant, from flower x-rayed two days after anthesis; showing bivalents of normal appearance, fragments both free and attached, univalents and altered univalents.

monly, however, they approximate in volume one-half the univalent of average size. It is clear that fragments are lost during meiosis in certain individuals because of the high percentage of small micronuclei or of diminutive microcytes containing small nuclei, at the tetrad stage. On the other hand, in many instances they are not so lost. Certainly they are not eliminated during mitosis since, for example, different P.M.C. from inflorescences on laterals arising during different portions of the growing season on different parts of the plant body may uniformly exhibit them. That they are usually maintained throughout gametogenesis of X_1 plants is shown by their presence in the original number and morphology in later generations (cf. Goodspeed and Avery, *loc. cit.*). There is a suggestion here that in such cases the fragments may represent portions of the chromosomal complement essential for gametic or zygotic viability, a sugges-

tion perhaps strengthened by the fact that chromosome survival in later generations is unrelated to initial chromosomal volume.

Significant evidence on various aspects of this problem of induced chromosome fragmentation should be obtained from studies of fragments in their somatic manifestation. Its relation to questions of chromosome structure and, particularly, the nature of the pellicle, is obvious, while the question of spindle fiber attachment in relation to constrictions and the distributional mechanism, calls for investigation.

CHROMOSOME "ADDITIONS"

This second type of induced chromosome alteration is frequent in occurrence, appears to be closely related to fragmentation, and undoubtedly represents a different end product of the process by which the latter is produced. The term "additions" or "fusions" is employed to refer to these end products and includes the more definitive categories of attachment of homologous or non-homologous chromosomes, of duplication, and of translocation. Such results of chromosome disruption and reorganization induced by x-rays are to be seen in the meiotic divisions of X_1 plants. At I-M there appear bivalents, apparently equivalent to those of the control in configuration, to which are added or with which are fused larger or smaller chromatin elements. When larger they usually are closely associated with the bivalent and the compound unit is then cytologically indistinguishable from a trivalent. Such a unit, however, differs from the latter in method of origin of its parts and, as contrasted with a trivalent, represents an instance of physical fusion not related to homology.

When, as is more frequently the case, the added chromatin elements are diminutive, involving duplication or translocation, they may be attached by a thread-like connection to the bivalent rather than closely fused with it (cf. fig. 1b). The added element is thus indistinguishable from a small fragment except that it possesses this tenuous union with a bivalent partner. The method of juncture involved here is not entirely clear and indicates the necessity of further evidence as to the nature of the superficial layer characteristic of mature chromosomes, and particularly of droplet-like chromosomes such as those of *tabacum*. The added chromatin element appears to maintain its identity throughout the meiotic divisions and, contained within its own pellicle, adheres to the pellicle of the entire chromosome to which it is united or maintains its contact therewith by means of the delicate thread.

That these attachments are of a permanent character and do not represent homologous fragments or univalents conjugated with a bivalent partner, is further apparent in their behavior at I and II-A. Thus, in place of exhibiting a characteristic separation from the remaining elements of a multivalent, they remain in the form of closely associated additions during I and II. Moreover, evidence from generations subsequent to X_1 shows that they are maintained in unaltered condition throughout mitosis and the ensuing meiosis. Very minute elements attached by a thread to a bivalent partner frequently occur also. They might be taken to be the product of deletion, a very small chromosome segment remaining associated with the major morphologically unaltered portion of the chromosome. Such chromosome alterations on the minus side probably survive less often than those, just described, on the plus side, even in *tabacum*, where minus deviations are more generally viable than in certain organisms with a smaller chromosome garniture.

NON-CONJUNCTION AND UNPAIRED CHROMOSOMES

These two types of induced alteration in X_1 of the typical content of I-M plates and meiotic chromosome behavior in *tabacum* are as closely interrelated as are the induced fragmentations and additions or fusions just described. The genic alterations, deletions or additions to various amounts which become characteristic of the chromosomes after x-radiation are responsible for a diminution in the closeness of conjugation, which is a feature of normal *tabacum* bivalents. Since a variety of causes may lessen this evidence of homology, it is not surprising that the presence of unpaired chromosomes is most characteristic of diakinesis and I-M of X_1 plants. As contrasted with the generally spherical, homogeneous bivalent of the control, "loose" conjugation may involve the production of a ring-like bivalent or conjugation at one end only. When the attraction is still weaker, the bivalent partners are found at I-M side by side, but never in contact. Presumably such association is a reflection of a loose conjugation during the meiotic prophase and it has been found in X_1 to be peculiarly characteristic of the smaller *tabacum* chromosomes and of those which have been reduced in volume as a result of fragmentation or deletion. A "loose" conjugation also occurs in the case of what appear to be "paired" fragments in X_2 and X_3 progenies (cf. Goodspeed and Avery, *loc. cit.*).

The ultimate condition occurs when there is a complete failure to pair or associate in any way on the part of I-M chromosomes. Usually this situation when followed by the presence of univalents, concerns only a few pairs of chromosomes, but in the case of two X_1 plants in

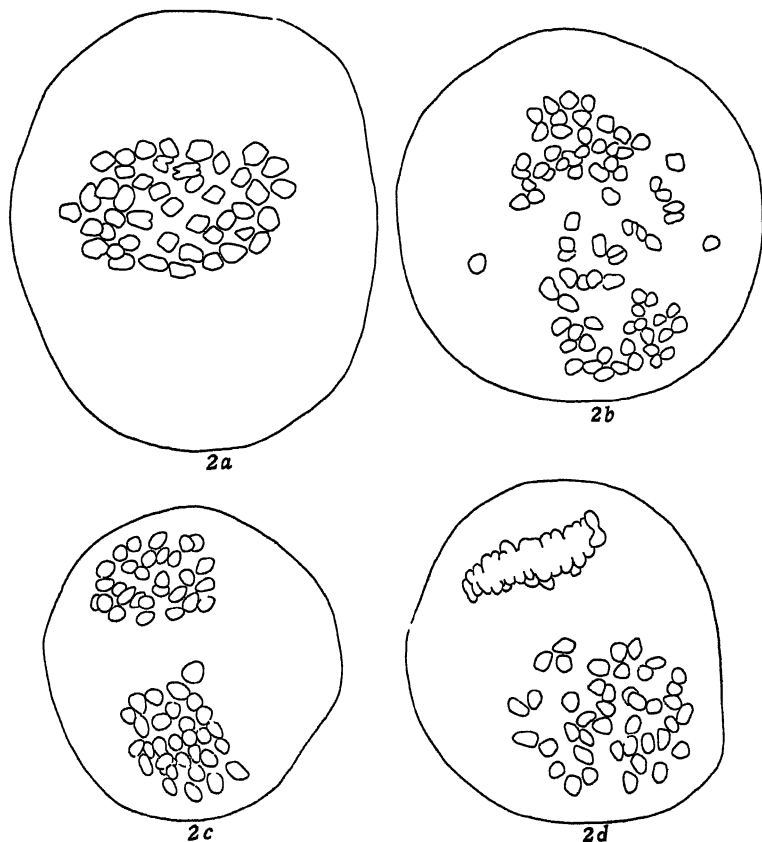


Fig. 2. 29313P₁₁, P.M.C., a, I-M showing forty-three units of which nine are bivalents; b, late I-A showing seventeen lagging univalents, six of which are dividing; c, II-M, chromosome number accurately determined in upper plate only where there are twenty-six units of which three show evidence of division; d, II-M, one plate in side view and the other in polar view, the latter containing forty-two units.

1927 and two in 1929, it was very extensive. The cytological evidence in this connection is shown in figures 2 and 3.

In 29313P₁₃ (figs. 2 and 3) rather regularly formed I-M plates occurred, but they contained both larger and smaller units whose total number varied from 28 to 47 but in most instances was from 40 to 45. In an I-M of 28 units there were $24_{II} + 4_I$ and in one with 43 units (fig. 2a) there were 9_{II} in addition to 34_I . In other words, the chromo-

some garniture of this X_1 plant was approximately 52 as contrasted with 48 for the control. Wide variations in I-A chromosome behavior in this individual are related to the relative amounts of bivalent formation from one P.M.C. to the next. Where most of the chromosomes have conjugated their distribution is quite regular, and II-M plates show from 20 to 30 chromosomes (fig. 2c) with some more advanced in the process of division than are the rest. More commonly larger numbers of univalents occur at I-M accompanied by considerable lagging and

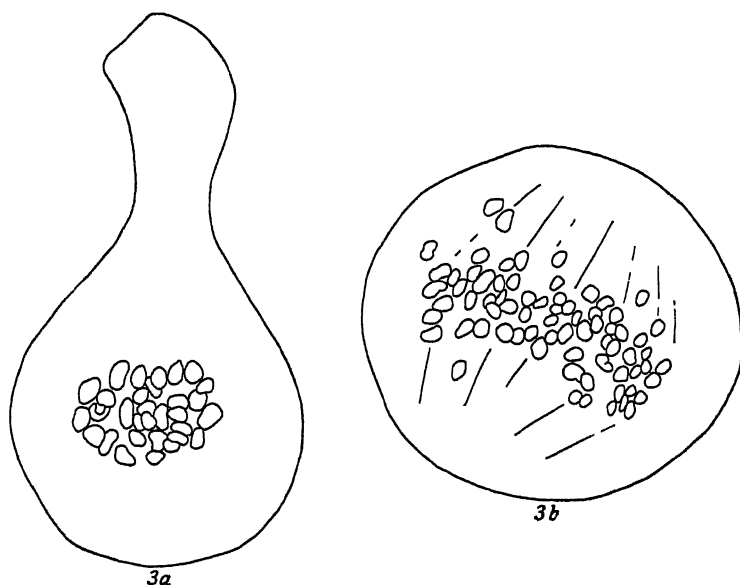


Fig. 3. 29313P₁₁, P.M.C. *a*, evidence of occurrence of suspended meiosis leading to condition shown in figure 3*b*; *b*, giant spindle containing more than eighty units.

univalent division at I-A. For example in figure 2*b*, six univalents are lagging and dividing while eleven additional laggards are present, representing undivided univalents or halves of univalents. At II-M in such cases there will be wide variation in number of units in the two plates. From 35 to 45 chromosomes have been seen at this stage, the most frequent number being 38. Figure 2*d* illustrates a case in which 42 units were included in a II-M plate. Here the smaller chromosomes presumably represent halves of univalents but their exact number cannot accurately be determined.

It is not easy to assign the large extent to which lack of pairing occurs in 29313P₁₂ to any one factor, because of the variation in the amount of conjunction at I-M of P.M.C., which it exhibits. As has

been indicated, certain chromosomes apparently are present in excess of their normal number so that genic balance may have become seriously disturbed. Thus the non-conjunction which occurs in this plant might be assigned to the presence of four or more supernumerary chromatin units. Again, there was evidence of induced quantitative alterations leading to chromosomal reorganizations, and to such conditions a certain proportion of the observed lack of pairing may be assigned. The striking alterations in the external morphology of the plant under discussion certainly suggested that both gene mutation and chromosome reorganization must have altered the characteristic condition of the hereditary material.

In some P.M.C. a very extensive division of unpaired chromosomes occurred at I, as shown in figure 3b. Here there is an unusually extended achromatic figure upon which are oriented more than eighty chromosomes, some of which are apparently undergoing still further division. A suspended meiotic division presumably precedes such a situation and what may be a preliminary in this case is shown in figure 3a. Here all the chromosomes are at one side of a large dumb-bell-shaped figure. Corresponding to such alterations in normal meiotic processes, there was a frequent occurrence of dyads at the tetrad stage.

In this X_1 plant II-A usually involves the presence of large numbers of lagging chromosomes, 12 to 18 occurring in each spindle. The majority were ultimately included in the granddaughter nuclei but a number were not, and gave rise to a considerable proportion of microcytes and micronuclei at the tetrad stage.

When such impairment of the capacity to conjugate is induced at gametogenesis, the X_1 progeny may be expected to contain additions to or subtractions from the normal chromosome garniture of *tabacum*. The most frequent classes under this category were monosomics and trisomics, i.e., $23_{II} + 1_I$ or $24_{II} + 1_I$. In certain cases, few in number, plants with a smaller number of pairs or with a larger number of univalents have been found to occur. Only a few of the individuals included in the former, more frequent classes resemble phenotypically the *tabacum* monosomics and trisomics known to occur in untreated lines (cf. Clausen and Goodspeed, 1924, 1926). Presumably, therefore, these same chromosomal types in x-rayed progenies involve an unbalance in chromosome sets which has not appeared or will not function in *tabacum* under normal conditions. In X_2 and X_3 in these cases the variant type is reproduced, in character expression

and chromosome garniture, with from 10 per cent to 15 per cent occurrence. In rare instances the morphology of basically monosomic or trisomic types is conditioned by other or additional chromosomal alterations or by associated transgenations simultaneously induced by x-radiation. Thus, for example, a series of types morphologically close to "fluted" *tabacum* (cf. Clausen and Goodspeed, 1926) but obviously involving more than the simple monosomic condition which it reflects, have been obtained. In general it is clear that, following x-radiation, a considerable series of monosomic and trisomic types can rapidly be obtained, presumably involving instances of chromosomal unbalance which would be found in untreated progenies only after a long period of observation, if at all.

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INTERSPECIFIC HYBRIDIZATION
IN NICOTIANA

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XI. THE CYTOLOGY OF A SESQUIDIPLOID HYBRID BETWEEN TABACUM AND SYLVESTRIS

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INTRODUCTION

The predominating influence of *Nicotiana tabacum* in determining the phenotype of F_1 *N. tabacum* (24_{II}) \times *N. sylvestris* (12_{II}) has been observed by numerous investigators. According to Goodspeed and Clausen (1917), the F_1 plants are replicas on an enlarged scale of the *tabacum* parent, but show some influence of *sylvestris*. Brieger (1928) found that *tabacum* dominated the hybrid in general habit, but that individual characters always showed a distinct influence of the other parent.

The explanation of the decided resemblance of the F_1 to *tabacum* has frequently been discussed. Christoff (1928) suggests that dominance of *tabacum* is probably due to the transmission by this species of a greater number of dominant factors along with the larger number of chromosomes. Brieger (1928) believes that *tabacum* dominance may be due to strict dominance over allelomorphs present in *sylvestris* or to a pseudo-dominance due to the lack of *sylvestris* allelomorphs. Watkins (1925) suggests that the *sylvestris* chromosomes may be genetically very similar to twelve of the *tabacum* chromosomes. If such is the case, the *sylvestris* chromosomes are sufficiently homologous with certain *tabacum* chromosomes to be combined with the *tabacum* set without disturbing the *tabacum*-like nature of the F_1 .

Watkins' assumption is based on Goodspeed and Clausen's (1917, 1922) F_1 and backcross data. These authors demonstrated that during meiosis the F_1 exhibits Drosera scheme behavior, there being $12t's + 12t^{cl}$.¹ In external morphology approximately 10 per cent of

¹ This chromosome formula aims to indicate the postulated origin via amphidiploidy of the two sets of twelve chromosomes each presented in the haploid set of modern *tabacum*. Thus " $12t^{cl}$ " indicate that the set of twelve *tabacum*, which is paired with the complete *sylvestris* set to produce " $12t's$," represents the contribution of the progenitor of modern *sylvestris* to the original F_1 hybrid. Similarly, the remaining set of twelve from *tabacum*, which occur as univalents, is designated as " $12t^{cl}$ " to indicate that it represents the contribution from the progenitors of modern *tomentosa*-like races which entered into the formation of this hybrid.

the *sylvestris* backcross plants approximate this species, and the remainder are abnormal in type; with *tabacum* pollen all backcross plants are analogous to *tabacum*, though exhibiting considerable variation. Goodspeed (1923) suggests that the F_1 functional eggs contain either 12 *sylvestris* or 24 *tabacum* chromosomes, but finds difficulty in assuming a chromosome distribution which would produce this result. Watkins believes it is much simpler to regard the two cytologically homologous sets (12t's) as being very similar to one another. Goodspeed and Clausen have shown that *sylvestris* derivative lines contain *tabacum* elements. This demonstrates that certain *tabacum* chromosomes can replace *sylvestris* homologues without greatly changing the *sylvestris*-like phenotype. Under such conditions the *tabacum* backcross plants resemble *tabacum* by reason of the 12 extra *tabacum* chromosomes (12t^v). The *sylvestris*-like backcross plants contain *sylvestris* chromosomes and genetically similar *tabacum* chromosomes; while the variant plants, in addition to these classes of chromosomes, contain one or more of the 12 *tabacum*-determining chromosomes (12t^v).

The present report upon a sesquidiploid² *tabacum* \times *sylvestris* hybrid and its progenies suggests some genetical and cytological considerations as to the nature of the interreactions of whole, parts, and duplicated parts of the chromosome complexes of these species and deals with possible methods of origin of new forms from such chromosomally unbalanced hybrids.

The male parent of this sesquidiploid hybrid was a *sylvestris* derivative from F_1 *tabacum* \times *sylvestris*. It possessed 12 pairs of chromosomes, of which, as indicated by genetical behavior, one *sylvestris* chromosome had been replaced by a *tabacum* homologues. The female parent was *tabacum* var. *purpurea*, with 24 pairs of chromosomes. Among a progeny of fifty F_1 plants, one highly fertile plant (26166P43) was observed. Aside from its striking fertility, 26166P43 differed only slightly in character expression from sister F_1 plants, which, as usual, were almost completely sterile. Its leaf-base was not of the F_1 intermediate type, but sharply constricted like *tabacum* var.

² In keeping with the term amphidiploid hybrid which refers to the occurrence of individuals whose chromosome garniture is diploid for both parents, the term sesquidiploid (sesqui=one and one-half) hybrid is proposed for a situation of the sort dealt with here, in which the F_1 is diploid for one parent and haploid for the other. Alternative terms, such as "triplex hybrid," do not appear to be so expressive of the condition obtaining. Although a "hybrid" term, it seems less cumbersome than such a more correct combination as "hemioliodiploid hybrid."

purpurea. The flower measurements also approach the *tabacum* parent (cf. table 1). Upon cytological examination, this plant showed $2n = 60$. Presumably, it arose from the union of a diploid *tabacum* (48) egg and a haploid *sylvestris* (12) pollen grain. During meiosis its PMC and EMC usually formed 24 bivalent and 12 univalent chromosomes and its suggested chromosome formula is $24tt + 12s$.

TABLE 1

FLOWER SIZE MEASUREMENTS OF A SESQUIDIPLOID *Nicotiana tabacum* \times *N. sylvestris* HYBRID, ITS PARENTS, AND ITS PROGENY

	Spread in mm	Length in mm
<i>tabacum</i> var. <i>purpurea</i>	36.00	49.3
<i>sylvestris</i>	42.00	85.3
F ₁ <i>sylvestris</i> \times <i>tabacum</i>	44.1	59.0
sesquidiploid hybrid.....	33.3	47.8
F ₁ sesquidiploid hybrid \times <i>tabacum</i> ...	26 to 44*	40 to 60
F ₁ sesquidiploid hybrid \times <i>sylvestris</i>	30 to 51	45 to 80
F ₂ sesquidiploid hybrid.....	26 to 48	40 to 60
F ₂ sesquidiploid hybrid (27G122).....	37.4	49.4

*Extreme limits of variation.

MATERIAL AND METHODS

The sesquidiploid hybrid occurred in 1926 and was maintained for several years by vegetative propagation. Frequent cytological examinations of these cuttings showed that they preserved their sesquidiploid nature. Selfed seed was easily obtained, which in nearly every case gave rise to highly fertile F₂ plants. The sesquidiploid hybrid produced seed abundantly when either *sylvestris* or *tabacum* pollen was used and both of these parental species were highly fertile with the sesquidiploid hybrid pollen. All plants of the *tabacum* backcrosses were highly fertile, while the *sylvestris* backcross plans were self-sterile and sterile with *sylvestris* pollen.

The sesquidiploid hybrid and its progenies were grown in the University of California Botanical Gardens and greenhouses under the following culture numbers:

SESQUIDIPOID HYBRID = 26166P43

F ₂ *	F ₃	F ₄	F ₅	F ₆
28264.....	{ 29289 (50) ** 29290 (7) 29291 (15) { 27G106 (11) 27G107 (18) 27G108 (12) 27G109 (17) 27G122 (16)	28267 (15).....	{ 28562 (3)..... 28563 (4) 28564 (13) 28565 (27)..... 28566 (29)	{ 29353 (15) 29354 (23) 29356 (9) { 29355 (6) 29357 (13)

*Since 26166P43 occurred in F₁, its immediate selfed progeny was designated as F₂.

**Numbers in parentheses denote the plant in the preceding generation from which each population is derived.

BACKCROSS POPULATIONS

28265 = 26166P43 ♀ × *N. tabacum* var. *purpurea* ♂

29286 = *N. tabacum* var. *purpurea* ♀ × 26166P43 ♂

29287 = 26166P43 ♀ × *N. sylvestris* ♂

In other words, selfed progenies of the original sesquidioid hybrid which was discovered in 1926 have been grown for five generations. In addition, the sesquidioid hybrid has been crossed with *tabacum*, both pollen and eggs, and with *sylvestris* as pollen parent. For convenience, the following terminology will be employed to designate these various progenies: *sd* for the sesquidioid hybrid (F₁*sd*, F₂*sd*, etc.), *t* for *N. tabacum* var. *purpurea*, and *s* for *N. sylvestris*.

The cytological observations were made from paraffin material, permanent smears, and aceto-carminic smears. The cut material was killed and fixed in chrom-acetic-formalin, made up and used as follows:

- A 65 cc. distilled water
 10 cc. glacial acetic acid
 1 gr. chromic acid

Mix one part A with one part B
 just before fixing.

- B 40 cc. formalin (commercial)
 35 cc. distilled water

Paraffin sections were cut 10 μ thick and stained in Haidenhain's iron-haematoxylin. This method usually gave very uniform preparations of root tips and of EMC, in ovules from which the ovary wall had been removed. The best PMC were obtained in anthers which had been dipped for a few seconds in Carnoy's fluid before placing in the chrom-acetic-formalin solution.

Oceto-carminic smears of PMC proved very satisfactory for obtaining chromosome counts at I-M and II-M. These slides, however, were much less intensely stained than either the permanent smears or cut material and were mainly used as a check against the other methods.

Permanent smears of PMC were made according to Webber (1929). Those stained with brazilin (Belling, 1928) proved to be the most satisfactory. In such slides, size differences and color tones of univalents, bivalents, trivalents, and quadrivalents are very clear. In the majority of cases the chromosomes were well separated and equal in definition to those in aceto-carminic smears. The results obtained from the three types of preparations are in perfect agreement.

Camera lucida drawings were corrected by direct observation, and finally recomposed by means of the camera lucida. The drawings are reproduced at 2000 diameters. No attempt has been made to show relative levels at which chromosomes lie. Photomicrographs are magnified about 800 diameters.

THE SESQUIDIPOID HYBRID

As already stated, this fertile plant which occurred in an F_1 *tabacum* \times *glauca* progeny in 1926 possessed sixty somatic chromosomes. Characteristic metaphase plates are shown in plate 10, figure 1 and text figure 1.



Fig. 1. F_1 sesquidiploid *N. tabacum* \times *glauca* (26166P_{ss}). Somatic plate showing 60 chromosomes.

During the diaphase and I-M in PMC and EMC there are three classes of chromosomes, trivalents, bivalents, and univalents. Table 2 gives the distribution of these classes and reveals the occurrence of one principal type of conjugation, viz., $24_{II} + 12_I$.

TABLE 2

FREQUENCIES OF CHROMOSOME CLASSES AT I-M IN *sd*

Types of conjugation	Frequency	Per cent of frequency
$4_{III} + 20_{II} + 8_I$	5	3.25
$3_{III} + 21_{II} + 9_I$	10	6.49
$2_{III} + 22_{II} + 10_I$	13	8.44
$1_{III} + 23_{II} + 11_I$	18	11.69
$24_{II} + 12_I$	108	70.13
Total.....	154	100.00

The bivalent chromosomes formed a well organized equatorial plate, which included any trivalents that were formed. The univalents were usually scattered over the spindle, figure 2. Rarely, however, was a plate observed, such as shown in plate 10, figures 2 and 3, where all



Fig. 2. F_1 sesquidiploid *N. tabacum* \times *sylvestris*. Typical I-M, profile view, showing scattered nature of univalents.



Fig. 3. F_1 sesquidiploid *N. tabacum* \times *sylvestris*. Typical I-M, polar view, $24_{II} + 12_I$.

classes of chromosomes were in nearly the same plane. A typical $24_{II} + 12_I$ plate is shown in figure 3, in which, while all chromosomes of this semidiagrammatic drawing appear to be in the same level, the univalents are actually scattered. In figure 4 is shown a PMC with $3_{III} + 21_{II} + 9_I$.

At the anaphase the bivalents disjoined normally and their partners moved toward opposite poles as single units. This disjunction included trivalents, one partner moving toward one pole and two partners toward the other. The bivalent from the trivalent soon disjoined and both partners continued toward the same pole. While



Fig. 4. F_1 sesquidiploid *N. tabacum* \times *sylvestris*. I-M, $3_{III} + 21_{II} + 9_I$.

the bivalent and trivalent partners were separating the univalents remained in a scattered condition. Immediately following disjunction a few univalents were found in the equatorial zone (pl. 10, fig. 4 and text fig. 5). These univalents were originally included in the I-M bivalent-trivalent plate. The remaining univalents, which were originally above or below the I-M plate, moved toward the poles along with the products of disjunction (fig. 5). Occasionally all the univalents were found in the equatorial region (fig. 6), giving the impression that the I-A distribution was in two successive stages. This condition presumably resulted from a I-M plate which included all the chromosomes, as explained above. The univalents left in the equatorial region

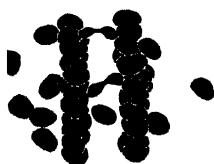


Fig. 5. F_1 sesquidiploid *N. tabacum* \times *sylvestris*. Typical early I-A, few univalents in equatorial region are presumably those which were intermingled in I-M bivalent plate. Remaining scattered univalents move toward the poles with the products of disjunction.



Fig. 6. F_1 sesquidiploid *N. tabacum* \times *sylvestris*. Late I-A, all univalents in equatorial region, four have nearly or completely divided. All univalents in this case presumably were intermingled in I-M bivalent plate.

became laggards and went through an apparent division (pl. 10, fig. 5, and text figs. 5 and 6). They were later distributed at random toward the poles, but on account of their delayed movement they were frequently left in the plasma. Now and then a laggard completed division (fig. 6), and the resulting halves were left in the plasma or included in the daughter nuclei.

The II-M plates were well separated and contained sharply distinct chromosomes. The counts of this stage are given in table 3. The mode was 30, as would be expected when univalents were distributed at random at I-A, while the mean value was 28.98. A typical II-M is shown in figure 7, depicting 30 + 30. While the total number of

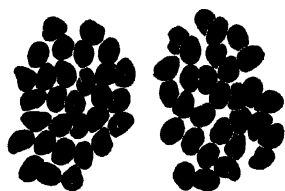


Fig. 7. F_1 sesquidiploid *N. tabacum* \times *sylvestris*. Typical II-M, 30-30.

chromosomes in these plates was 60, it was frequently below and occasionally above this number. Out of 34 II-M PMC in which both plates were countable, only three contained more than sixty chromosomes. They showed 29 + 32, 30 + 1 + 31, and 30 + 31, respectively. The exact composition of these plates could not be determined, since univalent halves were indistinguishable from other chromosomes at this stage. II-M conditions confirm the occurrence of occasional univalent division during I, and loss of univalents in the plasma.

TABLE 3
NUMBER OF CHROMOSOMES AT II-M IN F_1 sd^*

Type of distribution	24 36	25 35	26 34	27 33	28 32	29 31	30 30
II-M both plates.....	0 0	0 0	1 1	4 4	7 7	10 10	12 12
II-M single plates.....	6 0	14 0	20 1	35 5	43 15	48 39	37
Totals.....	6	14	23	48	72	107	61

*PMC exhibiting more than 60 chromosomes are not included.

At late II-A the following counts were obtained:

28	2	28	29	1	28	28	2	28	27	3	27	30	1	30
∧	∧	∧	∧	∧	∧	∧	∧	∧	∧	∧	∧	∧	∧	∧
2+		+2-	1+	1+	2-	1+		+2-	1+		+2-		+	+
∨	∨	∨	∨	∨	∨	∨	∨	∨	∨	∨	∨	∨	∨	∨
28	2	28	28	1	29	29	2	28	29	3	28	29	1	31

All but the last of these PMC total the expected 120 chromosomes. This contained 122 units and therefore involved the division of one univalent during I and the division or fragmentation of its halves during II. This PMC is shown in figure 8a, b. Four of its chromosomes were considerably smaller than the others. Whether these four would have regained their normal size at II-T could not be determined. As noted below, however, several plants in *sd* progenies have exhibited chromosome fragments, and it seems possible that such double division of univalents may explain their formation.

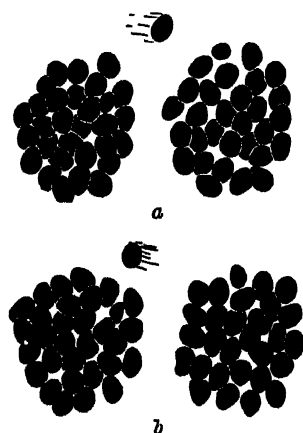


Fig. 8a, b. F₁ sesquidiploid *N. tabacum* × *syvestris*. Late II-A; a, upper two groups and b, lower ones in the same cell. Distribution

30	1	30
∧	+	∧
29	1	31

The figure necessitates the division of one univalent during I and the division of its halves at II. One small chromosome is distinguishable in each group.

In the first four II-A figures given above, an average of 7.5 chromosomes was left in the plasma. Since during I, 2.04 (17 per cent) of the univalents were eliminated, during II, 1.71 (14 per cent) were eliminated. This percentage of elimination is in agreement with the distribution of univalents found in the backcross progenies described below. Presumably these eliminated chromosomes were of *syvestris* origin, which would mean that the gametes received an average of 4.13 chromosomes of this species.

At the completion of meiosis 38.55 per cent of the tetrads contained four microspores, 50.67 per cent exhibited microcytes, and 17.84 per cent exhibited micronuclei.

SESQUIDIPOID HYBRID (F_1 *sd*) \times *tabacum* (*t*)

A population of fifty plants was in each case grown from $sd\bar{\sigma} \times t\bar{\sigma}$ and $t\bar{\sigma} \times sd\bar{\sigma}$. Because of the striking similarity of these populations in external morphology and in cytological behavior, they will be discussed together. In external morphology they approached *t*, but exhibited numerous minor distinctions from it and among themselves. These differences were mainly concerned with habit of branching (pl. 11); leaf shape and size (pl. 14, figs. 11–13); and flower size and shape (pl. 15, figs. 7 and 8, and table 1). All plants were highly fertile, with flowers of carmine, *tabacum*, color. An attempt to classify and divide these plants into types met with failure. The relation between the number of chromosomes present and the various phenotypic expressions could not be determined.

The meiotic behavior in these plans was practically the same as that in *sd*. The *tabacum* chromosomes conjugated, forming $24tt$. The *sylvestris* chromosomes remained as univalents. Typical I–M plates are shown in plate 10, figures 6 and 7, depicting $24_{II} + 5_I$, and $24_{II} + 9_I$ and figure 9, depicting $24_{II} + 3_I$. Occasionally trivalents were formed,



Fig. 9. F_1 sesquidiploid *N. tabacum-sylvestris* \times *tabacum* (28265P₆).
Typical I–M, $24_{II} + 3_I$.

the partners of which disjoined before they reach the poles. The bivalent partners were distributed normally and the univalents exhibited a random distribution, although an apparent division was occasionally seen. The homotypic division was rather regular, even though a few chromosomes often lagged. Tetrads, in addition to the four major cells, often contained a few microcytes and micronuclei.

The chromosome numbers determined in the plants of these populations are recorded in table 4.

TABLE 4

FREQUENCIES OF CHROMOSOME NUMBERS IN PROGENIES OF $sd \times t$. ALL POSSESS $24_{II} + n_I$, n HAVING VALUES FROM 1 TO 9*

Values of n	1	2	3	4	5	6	7	8	9	Totals
$sd \text{♀} \times t \text{♂}$	2	1	4	6	7	5	3	3	1	32
$t \text{♀} \times sd \text{♂}$	2	3	5	7	5	3	0	1	0	26
Totals	4	4	9	13	12	8	3	4	1	58

*Table 4 does not include a single instance of $23n+6t$

An examination of the data contained in table 4 shows that the 58 plants examined belong to the series $24_{II} + n_I$, where the value of n ranged from 1 to 9. The average value of n in the $sd \text{♀} \times t \text{♂}$ plants was 4.96; in the $t \text{♀} \times sd \text{♂}$, 3.92, and in the two populations combined, 4.50. The latter figure is only 75 per cent of the expected value six, based on purely random distribution of the twelve sd univalents. The results therefore indicate either that univalents were eliminated in sd during meiosis or that certain gametes or zygotes classes did not function. The observations recorded above in the case of sd indicated that chromosome elimination is involved in this situation.

The few counts made during late II-A showed that the gametes contained an average of 4.13 *sylvestris* chromosomes. This is in close agreement with the average, 4.50 univalents, in these $sd \times t$ plants.

That chromosome elimination took place in sd was also indicated by the high percentage of microcytes and micronuclei which appeared at the tetrad stage. The result of the reciprocal cross ($sd \times t$) showed that the percentage of inviability of gametes and zygotes was low. In $sd \text{♀} \times t \text{♂}$, 83 per cent of the expected six univalents were obtained. This would indicate that nearly all zygotes and female gametes were functional. Furthermore, this number is above the observed distribution of *sylvestris* chromosomes (4.13) in sd . Moreover, all progenies of sd have given evidence that nearly all gametes and all zygotes containing the complete *tabacum* haploid, and especially the diploid chromosome set, were viable. On the other hand, in the $t \text{♀} \times sd \text{♂}$ progeny only 66 per cent of the expected six univalents occurred. It would appear in this case that there was a low transmission of extra chromosomes through the male gametes. In the progenies of the sd hybrid, therefore, the failure to obtain six *sylvestris* chromosomes from each gamete was mainly due to chromosome elimination and possibly slight inviability of male gametes.

Besides the plants listed in table 4 one exceptional individual occurred. This plant uniformly exhibited $23_{II} + 6_I$ at I-M. Disjunction of *tabacum* homologues (*tt*) from a trivalent (*tts*) formed during meiosis in the *sd* parent may account for its origin. On this assumption the gamete was of the constitution $23t + 5s$, and, upon union with $24t$, gave rise to the individual which during meiosis formed $23tt + 1t + 5s$. It is clear, with such disjunction of *tabacum* homologues from trivalents formed in the *sd* parent, that an occasional univalent, recorded in table 4, must have been *t* rather than *s*. Likewise, a few of the bivalents were probably *ts* rather than *tt*. Further evidence in support of such replacement of *tabacum* and *sylvestris* homologues is to be found in the case of the selfed *sd* progeny discussed below.

SESQUIDIFLOID (F_1 *sd*) \times *sylvestris* (*s*)

The fifty plants grown from the cross $sd\text{♀} \times s\text{♂}$ were sterile, and were so highly variable that no attempt was made to classify them into types. The extreme variations in habit of growth and in leaf and flower characters are to be seen in plate 12, figures 1-3, plate 14, figures 8-10, plate 15, figures 5 and 6, and table 1. The photographs clearly show that the population as a whole was intermediate between *tabacum* and *sylvestris*. The individual plants, however, approached toward one or the other of these species. The relation between phenotypic expression and the number of *sylvestris* and *tabacum* chromosomes is discussed later.



Fig. 10. F_1 sesquidiploid *N. tabacum-sylvestris* \times *sylvestris* (28287P₄₁).
Typical I-M, $6_{III} + 6_{II} + 12_I$.

During diaphase and I-M these plants always showed twenty-four chromosomes. Twelve of these units were *tabacum* univalents. The other twelve were bivalents and trivalents, of the composition *ts* and *tss*. In figure 10 is shown a I-M with $6_{III} + 6_{II} + 12_I$. This plate was presumably of the constitution $6tss + 6ts + 12t$, the plant having received from *sd* 24 *tabacum* plus 6 *sylvestris* chromosomes. The distribution of chromosome classes at I-M in the plants studied is given

in table 5. This table also includes the calculated frequencies of *sylvestris* chromosomes received from the *sd* parent. It must be remembered that in several of the progenies there was evidence of replacement of *tabacum* and *sylvestris* homologues. In such a case, then, it is possible that some of the trivalents and bivalents listed in table 5 may have been of the constitution *its* and *ss* instead of *tss* and *ts*.

TABLE 5

FREQUENCIES OF CHROMOSOME NUMBERS IN PROGENIES OF *sd* × *s*. ALL POSSESS $12_I + x_{III} + (12 - x)_{II}$, *x* HAVING VALUES FROM 1 TO 7

Frequency	Trivalents (<i>tss</i>)	Bivalents (<i>ts</i>)	Univalents (<i>t</i>)	Number of <i>sylvestris</i> chromosomes from <i>sd</i>
1	1	11	12	1
1	2	10	12	2
2*	3	9	12	3
4	4	8	12	4
2	5	7	12	5
1	6	6	12	6
1	7	5	12	7

*In one of these two plants a chromosome fragment was also seen.

In these plants the number of *sylvestris* chromosomes transmitted through the *sd* parent ranged from 1 to 7, with an average of 4. This average agrees fairly well with the 4.13 distribution observed in the *sd* hybrid and the 4.50 univalent average in the *sd* × *t* progenies. It therefore confirms the apparent occurrence of univalent or *sylvestris* elimination during I and II in *sd* itself.

In table 5 one exceptional individual is listed which, in addition to the normal chromosome classes, possessed a chromosome fragment. This fragment was about one-half the size of the average univalent. It was probably, as suggested above, the result of a division of a univalent in I and fragmentation of its halves during II. Usually the fragment was attached to a bivalent, in which case the whole unit behaved as did the trivalent. Occasionally, however, it remained separate and behaved as did the univalents.

During I-A and interkinesis, the chromosomes of these plants corresponded in behavior to that of progenies previously discussed. In the homotypic stages, however, irregularities leading to diad and triad

formation occurred. These irregularities have been discussed by Webber (1930) and will be dealt with in a subsequent report which will take up in greater detail this $sd \times s$ progeny. Here it may be said that the fusion of II achromatic figures and the suspension of II-A distribution are apparently involved.

At the completion of meiosis, 94 per cent of the PMC had formed tetrads, 5 per cent had formed diads, and 1 per cent, triads. About 53 per cent were normal and 47 per cent contained microcytes and micronuclei. In other words, as far as chromosome numbers were concerned, 2.4 per cent of the gametes were approximately diploid and 97.6 per cent haploid.

The significance of the cytological evidence submitted in the preceding pages will be discussed in the concluding section. It might be noted here that the extent and nature of the pairing observed in $sd \times t$ and $sd \times s$ confirms the assumed formula of $sd-24tt + 12s$ —and that meiotic behavior in all three cases was closely similar.

SELFED PROGENIES OF THE SESQUIDIPLOID HYBRID

As already indicated a number of generations have been grown and studied from sd selfed, in addition to those produced from crossing it with t and s which have been described above. A total of 75 plants (F_2) was grown from selfed seed of sd . These plants were vigorous and with a few exceptions were highly fertile. Although the populations resembled *tabacum* the individual plants showed considerable variation and in a few cases were intermediate between *tabacum* and *sylvestris*. In plate 13, figures 1 and 2, plate 14, figures 3-7, plate 15, figures 9-12, and table 1 are shown the extreme variations in habit of growth and leaf and flower characters exhibited. The chromosome numbers of the plants studied in this progeny are recorded in table 6.

Table 6 indicates that during meiosis from 24 to 29 bivalents and from 1 to 8 univalents were formed. A typical I-M plate is shown in figure 11 where there were $28_{II} + 4_I$. Since each gamete giving rise to these plants contributed only 24 *tabacum* chromosomes, all chromosomes above 48 were presumably *sylvestris*. In table 6 the calculated number of *sylvestris* chromosomes is also given. The 18 plants studied received 144 *sylvestris* chromosomes, or an average of 8 each. This would mean that each gamete contributed to the plants an average of 4 *sylvestris* chromosomes.

TABLE 6

FREQUENCIES OF CHROMOSOME NUMBERS IN F_1 *sd*. ALL POSSESS $k_{II} + n_I$, k HAVING VALUES FROM 24 TO 29 AND n FROM 1 TO 8

Value of k	Values of n								Number of <i>sylvestris</i> chromosomes
	1	2	3	4	5	6	7	8	
24	1			1	1	1	1		23
25		1	1				1		18
26			1	1			1	1	38
27				2					20
28	1	1		1					31
29				1					14
	Total <i>sylvestris</i> chromosomes								144

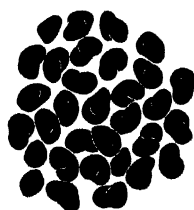


Fig. 11. F_1 sesquidiploid *N. tabacum* \times *sylvestris* (26G209P₁₂). Typical I-M, $28_{II} + 4_I$.

These plants occasionally formed trivalent and rarely quadrivalent units. The elements of quadrivalents, trivalents, and bivalents were disjoined and distributed normally during I. With the exception of slight lagging and elimination, the univalents were distributed at random. The homotypic divisions were rather regular, though a few chromosomes often lagged and were occasionally left in the plasma. The tetrads of individual plants exhibited considerable variation in number of microcytes and micronuclei.

The formation of varying numbers of bivalents is precisely what one would expect if the chromosome formula of *sd* was $24tt + 12s$, as has been assumed throughout this paper. In these plants every bivalent above 24 was composed of *sylvestris* homologues and all univalents were non-homologous *sylvestris* chromosomes. The chromosome complement shown in figure 11 was therefore of the constitution $21tt + 4ss + 4s$.

TABLE 7
SEGREGATION IN F_2sd (26G208P₁₀) PROGENIES

F_2	F_4	F_3	F_5
<p>27G122 Character—F_2 carmine and F_3 pink Cytology* of F_2 carmine $P_{15} = 25n + 2t$.....</p>			
<p>28267 Character—F_2 carmine and F_3 pink Cytology of F_2 carmine $P_3 = 26n; 10\%^{**}$</p>		<p>28562 Character—F_2 carmine Cytology—$P_9 = 24n + 1t; 11\%$ $P_{10} = 26n; 12\%$ $P_{13} = 25n + 1t; 13\%$</p>	<p>29356 Character—F_2 carmine Cytology—$P_8 = 24n; 4\%$ $P_{10} = 24n + 1t; 14\%$ $P_{12} = 24n; 1\%$</p>
	<p>$P_{12} = 26n; 13\%$.....</p>	<p>28564 Character—F_2 carmine and F_3 pink Cytology of F_2 carmine $P_9 = 26n ?$ Cytology of F_3 pink $P_{13} = 26n + 1t$</p>	
	<p>$P_4 = 25n + 1t; 21\%$.....</p>	<p>28563 Character—F_2 carmine and F_3 pink</p>	
	<p>Cytology of F_3 pink $P_{27} = 26n; 14\%$.....</p>	<p>28565 Character—F_3 pink Cytology—$P_6 = 25n; 8\%$.....</p>	<p>29355 Character—F_3 pink Cytology—$P_2 = 24n + 1t; 11\%$ $P_8 = 25n; 2\%$ $P_{29} = 25n; 4\%$</p>
	<p>$P_{39} = ?; 23\%$.....</p>	<p>28566 Character F_3 pink</p>	

*Only the more common type of conjugation is recorded. All plants except those F_4 individuals showing $24n$ and $26n$, occasionally formed trivalents and quadrivalents.
**Percentage of microcytes. About 4.5 per cent are formed during meiosis in *N. tabacum* var. *purpurea*.

Since *sylvestris* homologues pair in the F_2 plants, it should be possible to build up many new, constant races by selfing. Theoretically these races should possess from 25_{II} ($24tt + 1ss$) to 36_{II} ($24tt + 12ss$). As replacement occurs, however, an occasional bivalent might be *ts* rather than *tt* or *ss*. In the progenies of plants where replacement occurs, the other *tabacum* homologue may also be replaced or duplicated. It is therefore possible in the segregating generations of F_2 *sd*, to obtain plants with more or less than 24 *tabacum* pairs. However, since the loss of *tabacum* chromosomes is due to *sylvestris* replacement, no plants exist with less than 24 pairs. In an attempt to establish such constant races, a number of later generations have been grown from several of the original F_1 plants. Since these segregating generations exhibited similar genetical and cytological behavior, the lineage of only one plant, 26G209P₁₀, will be discussed.

26G209P₁₀ formed $25_{II} + 3_I$ at I-M and was a large, vigorous, highly fertile plant. In external morphology it exhibited very little, if any *sylvestris* influence. In habit of growth it was like *tabacum*. The leaves showed the ovate blade, constricted base, and broad-winged petiole characteristic of *tabacum* var. *purpurea*. Although the flowers resembled this *tabacum* variety in shape and carmine color, they were slightly smaller. The calyx lobes were unlike those of either *sylvestris* or var. *purpurea* in that they were long and sharply acute.

In the subsequent generations from 26G209P₁₀, two types of plants occurred. One exhibited the *sylvestris* spatulate-oblong leaf, with broad clasping base, and bore pink flowers. Since this type first occurred in F_1 and all other pink flowered plants in lineage were equivalent to it, the type will be referred to as F_2 pink (pl. 15, fig. 3). All other plants of this lineage were morphologically equivalent to the F_2 parent and will be referred to as F_2 carmine (pl. 15, fig. 4). Segregation in 26G209P₁₀ progenies is briefly summarized in table 7.

The F_0 , 24_{II} plants did not form quadrivalents or trivalents. They exhibited normal cytological behavior throughout meiosis and produced normal tetrads. Apparently these plants contained two homologous chromosome sets which were composed of genetically different chromosomes. Unquestionably they have produced a constant race decidedly different from either *N. tabacum* var. *purpurea* or *sylvestris*. Although the F_0 , 25_{II} plants likewise exhibited normal cytological behavior, it is still a question whether they constitute constant races. These segregating generations and other populations of *sd* gave sufficient evidence for the belief that chromosome elimination and replace-

ment is directly related to quadrivalent, trivalent, and univalent formation. Any one of these types of chromosomes has been shown to be formed when four homologous chromosomes are present, as is the case in these plants. In any event, only two of the four homologues will be lost, and a 24-paired race similar to or identical with the F_0 parent should be established.

On the other hand, from the fact that the *sd* hybrid never exhibited more than four trivalents, it seems possible that some combination has been obtained in which *sylvestris* homologues only pair *inter se*. Hence, the regular and constant cytological behavior of these plants indicates that they will breed true for chromosome number as well as phenotype.

DISCUSSION

Since sesquidiploid hybrids are chromosomally unbalanced, they are incapable of sexual reproduction without segregation. They are therefore a source of new chromosome combinations. Many of these combinations have been obtained in later selfed and backcross progenies of *sd*. Those in the immediate selfed and backcross plants give some evidence as to the genetic nature of *tabacum* and *sylvestris*, which has frequently been discussed in connection with the F_1 hybrid between them (cf. pp. 1-4). The external morphological characters and probable chromosome composition of the *sd* populations are briefly as follows:

sd = (24tt + 12s); *tabacum* type, leaf base intermediate.

sd x *t* = (24tt + 4s); *tabacum* type, minor phenotypic variations

sd x *sd* = (24tt + 4ss); *tabacum* type, considerable phenotypic variations, occurrence of intermediate characters.

sd x *s* = (24t + 4ss + 8s) intermediate type, considerable variation, *tabacum* and *sylvestris* characters.

Since intermediate characters are exhibited in plants which contain only a few *sylvestris* chromosomes in addition to those of *tabacum*, it is clear that *tabacum* dominance in *sd* is due neither to a greater number of *tabacum* chromosomes nor a lack of *sylvestris* allelomorphs. One might assume that the marked resemblance of F_1 *tabacum* x *sylvestris* and *sd* to *tabacum* was due to the latter's dominance over a balanced system of many interacting factors. Such a balanced system, however, would be destroyed in the *tabacum* backcross and in the selfed progenies, so that the factors carried by the individual *sylvestris* chromosomes should react to a greater extent with those of *tabacum* in determining the phenotype. The large amount of variation in the

sd selfed progenies suggests that this might be the case. On the other hand, the *sd* \times *t* plants are strictly of *tabacum* type, and exhibit only slight phenotypic variations. Presumably, if such a balanced system were operating, the phenotypic variations in these populations should be equivalent, since chances for destruction or retention of a balanced system were equal. Hence, the much greater uniformity in the *sd* \times *t* progenies indicates that such a system is not operating.

If we assume that dominant factors are included in the *tabacum* chromosomes which conjugate with those of *sylvestris*, the phenotypic variations characteristic of *sd* progenies are readily explained on the basis of replacement and duplication of these homologues. Goodspeed and Clausen's (1922) backcross data, however, cannot be interpreted in the light of such an assumption. *Sd* results may be harmonized with F_1 *tabacum* \times *sylvestris* and backcross data if Watkins' (1925) suggestion (cf. pp. 1-2) is accepted in part. It is inapplicable if it is assumed that the *sylvestris* chromosomes are completely genetically equivalent to the twelve *tabacum* homologues, unless it is also assumed that some of the non-homologous *tabacum* (*tabacum* determining 12t^u) chromosomes are lost. It seems more likely that the genetic similarity of *sylvestris* chromosomes to certain *tabacum* chromosomes is not complete and that several dominant factors are contained in a few of the *tabacum* F_1 homologues. In the selfed progenies of *sd* a complete pair or only one *tabacum* homologue may be replaced, while in *sd* \times *t* a complete pair is never replaced. Hence, phenotypic variations due to extra or duplicated *tabacum* chromosomes were visible in both selfed and *sd* \times *t* populations. However, a few recessive *sylvestris* characters were only visible in the selfed progenies. Variations in the *sd* \times *s* populations were due to duplication and replacement of *tabacum* chromosomes, and to duplication of *sylvestris* chromosomes. In some of these progenies *sylvestris* chromosomes were probably replaced by *tabacum* as well as *tabacum* by *sylvestris*. It is very likely that chromosome replacement was more frequent than has been assumed throughout previous sections of this paper.

As stated above, *sesquidiploid* hybrids are chromosomally unbalanced and a source of further alterations. In the progenies of *sd* many new chromosome combinations were obtained and several constant races possessing these combinations have been established. The cytogenetic behavior of the generations leading to the establishment of these races further supports the suggested genetic similarity of the *sylvestris* chromosome set to twelve of *tabacum* and replacement of a

few *tabacum* homologues which contained dominant factors. The important features of the segregating generations of F_2 26G209P₁₆ given above, are briefly summarized below.

F_2	F_3	F_4	F_5	F_6
25 _{II} +3 _I carmine	25 _{II} +2 _I carmine	$\left\{ \begin{array}{l} 26_{II} \text{ carmine} \\ 26_{II} \text{ carmine} \\ 26_{II} \text{ pink} \end{array} \right.$	$\left\{ \begin{array}{l} 24_{II}+1_{II} \text{ carmine} \\ \text{carmine and pink} \\ 25_{II} \text{ pink} \end{array} \right.$	$\left\{ \begin{array}{l} 24_{II} \text{ carmine} \\ \text{carmine and pink} \\ 25_{II} \text{ pink} \end{array} \right.$

With the exception of the F_4 plants which were selected on a purely cytological basis, these plants show a gradual elimination of chromosomes. A comparison of plant measurements, photographs, and notes showed that F_6 carmine flowered plants were equivalent to the F_2 parent. A similar comparison showed that F_6 pink flowered plants were like the F_3 pink flowered type. From F_2 to F_6 all combinations of the five chromosomes eliminated from F_2 to F_6 carmine type must have been obtained. Apparently these five chromosomes have very little, if any, effect on the phenotype. On the other hand, the elimination of three chromosomes from F_2 to F_6 pink type greatly changed the phenotype. From these results it is clear that one or several of the latter three chromosomes were not among the five eliminated in the first case. Since F_2 contained only five more chromosomes than normal *tabacum*, some of the *tabacum* chromosomes must have been eliminated in this lineage and replaced by *sylvestris*.

Several other lineages of *sd* have given similar results. F_2 28264P₁₆ contained 52 somatic chromosomes and gave rise to plants possessing from 49 to 52 chromosomes. Although these plants exhibited several minor distinctions in external morphology, they were practically equivalent to the F_2 parent. The fact that the majority of eliminated chromosomes had only slight, if any, effect on the phenotype supports Watkins' suggestion. On the other hand, F_2 28264P₇ and P₅₀ gave rise to F_3 generations which showed considerable phenotypic variation. Although the chromosome numbers of these plants have not been determined, undoubtedly chromosome elimination was responsible for this variation. Such decided phenotypic changes indicate a lack of complete similarity in F_1 homologues.

The exchange of homologous chromosomes between two species is by no means novel. It has been commented upon by Kihara (1924) and Goodspeed and Clausen (1927). The replacement of some *Nicotiana rustica* chromosomes by *N. paniculata* homologues apparently was responsible for the production of the new *rustica* forms obtained by East (1921). Goodspeed and Clausen suggest the replacement of

tabacum by *sylvestris* homologues to account for pink flowered plants in F_1 *N. sylvestris-tabacum* \times *sylvestris*. The genetical behavior of carmine and pink flowered plants in the F_2 26G209P₁₆ lineage, given above, indicates a similar exchange of *sylvestris* and *tabacum* homologues.

In the preceding section of this paper, it was shown that F_0 24— and 25—bivalent plants contained both *tabacum* and *sylvestris* chromosomes. In these derivatives cytological behavior was as regular and the fertility as high as in a pure species. The plants were equivalent to either *tabacum* or *sylvestris* in vigor of growth reaction and to this extent would compete with these species in a natural state. The 24_{II} plants would probably be considered a variety of *N. tabacum*, since they bear strong resemblance to this species. The 25_{II} plants likewise resembled *N. tabacum*, but exhibited several *sylvestris* characters. Since they exhibited a new chromosome number, as well as a new and distinctive character complex, they might receive more distinctive taxonomic recognition. These new races if isolated from each other and *N. tabacum* would undoubtedly perpetuate themselves. Isolation from *sylvestris* probably would not be so important, as the F_1 hybrid would be sterile.

On account of the affinity between *sylvestris* and *tabacum* chromosomes, it is doubtful whether races containing more than 24_{II} could have been established in nature from the spontaneous occurrence of a sesquidiploid *tabacum* \times *sylvestris* hybrid. However, if the original sesquidiploid hybrid had in some manner become isolated from *tabacum* eventually, through replacement of *tabacum* chromosomes by *sylvestris* and elimination of extra *sylvestris* chromosomes, a new 24-paired race would have been established.

Obviously any of the existing varieties of *N. tabacum* may have arisen from a sesquidiploid *tabacum* \times *sylvestris* hybrid, in a manner analogous to the 24_{II} plants described above. The same method of origin may possibly hold true for the varieties of other species of *Nicotiana* and even for the origin of not too distantly related species of the genus.

The 25-paired forms produced, illustrate methods by which species containing one or more extra pairs of chromosomes may originate. The F_0 24-paired plants demonstrate the possible origin through hybridization of new varieties and even new species containing the same chromosome number as that characteristic of one of the original parents of the hybrid.

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The writer gratefully acknowledges his indebtedness to Professor T. H. Goodspeed for suggestions and criticisms during the work. He is also indebted to Dr. I. E. Webber for advice in preparation of this manuscript.

SUMMARY

1. Cytological and genetical evidence indicate the occurrence in an F_1 *Nicotiana tabacum* \times *N. sylvestris* hybrid of an individual produced from the fertilization of a diploid *tabacum* (t) egg with a haploid *sylvestris* (s) pollen grain.

2. The plant was highly fertile, but differed only slightly in external morphology from sister plants, which were haploid for both parental species. It was shown to be $2n = 60$ and exhibited $24_{II} + 12_I$ at I-M, with the formula $24tt + 12s$.

3. The term sesquidiploid hybrid is suggested for individuals of this chromosome constitution.

4. Seventy per cent of the EMC and PMC of this plant formed $24_{II} + 12_I$, the remaining 30 per cent formed from 1 to 4 trivalents (tts). The bivalents and univalents behaved similarly to those of other *Nicotiana* hybrids exhibiting Drosera scheme behavior. Although *tabacum* homologues of tts usually passed to opposite poles, occasionally they were distributed to the same pole.

5. Late II-A counts showed that the gametes of this sesquidiploid hybrid received from 3 to 7, with an average of 4.13 *sylvestris* chromosomes in addition to 24 *tabacum* chromosomes.

6. The F_1 of the plants, sesquidiploid hybrid \times *tabacum*, belonged to the series $24_{II} + n_I$, where n ranged from 1 to 9 with an average value of 4.5. The plants occasionally formed trivalents.

7. The F_1 of the plants sesquidiploid hybrid \times *sylvestris* belonged to the series $12_I + x_{III} + (12-x)_{II}$ where x ranged from 1 to 7, with an average value of 4.0. In this progeny somatic gametes were formed by (a) fusion of II achromatic figures, and (b) suspension of II-A distribution.

9. a. The F_2 sesquidiploid hybrid plants belonged to the series $k_{II} + n_I$, where k ranged from 24 to 29 and n from 1 to 8. The plants occasionally formed multivalents.

9, *b*. Cytogenetic studies were made of selfed progenies of the sesquidiploid hybrid over five generations.

10. Selfed progenies of the sesquidiploid hybrid in later generations exhibited considerable variation in external morphology. Evidence is presented that this variation is largely a reflection of duplication of homologous *tabacum* chromosomes and replacement of *tabacum* by *sylvestris* homologues.

11. Owing to multivalent formation the segregating generations of F_2 mainly reverted to 24-paired plants. Owing to chromosome replacements, these plants contained elements of both *tabacum* and *sylvestris*. The new forms resembled *tabacum*.

12. F_3 25-paired plants exhibited normal cytological behavior and probably represent stable combinations. Although they resembled *tabacum*, they exhibited several *sylvestris* characters.

13. Neither of these new forms would survive under natural conditions unless isolated from *N. tabacum*.

14. If in nature the original sesquidiploid hybrid had in some manner become isolated from *tabacum*, a new 24-paired race could have been established. It is doubtful whether races containing more than twenty-four pairs could be formed under similar conditions.

15. Many existing varieties and closely related species of *Nicotiana* may have arisen through hybridization in a manner analogous to that by which these new 24- and 25-paired forms have been produced.

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EXPLANATION OF PLATES

PLATE 10

Fig. 1. F_1 sesquidiploid *N. tabacum* \times *sylvestris* (26166 P_{4a}) Somatic plate showing 59 chromosomes.

Fig. 2. F_1 sesquidiploid *N. tabacum* \times *sylvestris*. I-M, $24_{II} + 12_I$; all chromosomes lie in nearly the same plane.

Fig. 3. F_1 sesquidiploid *N. tabacum* \times *sylvestris*. I-M, $1_{III} + 23_{II} + 11_I$; all chromosomes lie in nearly the same plane.

Fig. 4. F_1 sesquidiploid *N. tabacum* \times *sylvestris*. I-A, lagging univalents.

Fig. 5. F_1 sesquidiploid *N. tabacum* \times *sylvestris*. Late I-A, dividing laggards.

Fig. 6. F_1 sesquidiploid *N. tabacum-sylvestris* \times *tabacum* (28265 P_9). I-M, $24_{II} + 6_I$.

Fig. 7. F_1 sesquidiploid *N. tabacum-sylvestris* \times *tabacum* (28265 P_9). I-M, $24_{II} + 9_I$.

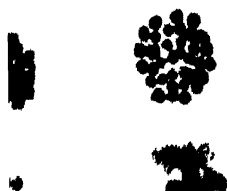
Fig. 8. F_1 sesquidiploid *N. tabacum-sylvestris* \times *sylvestris* (28267 P_{4a}). I-M, $6_{III} + 6_{II} + 12_I$.



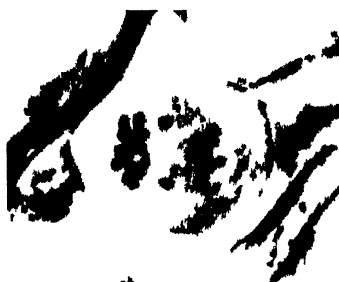
1



2



3



4



5



6



7



8

PLATE 11

Fig. 1. F₁ sesquidiploid *N. tabacum-sylvestris* × *tabacum* (29286P₁). A typical plant.

Fig. 2. F₁ sesquidiploid *N. tabacum-sylvestris* × *tabacum* (29286P₂). Extreme variant in a population of fifty.



PLATE 12

Figs. 1 and 3. F_1 sesquidiploid *N. tabacum-sylvestris* \times *sylvestris* (29287P_{23, 24}). Extreme variants in a population of fifty.

Fig. 2. F_1 sesquidiploid *N. tabacum-sylvestris* \times *sylvestris* (29287P₂₃). A typical plant.



PLATE 13

Figs. 1 and 2. F₂ sesquidiploid *N. tabacum* × *syilvestris* (28264P₂, ss). Extreme variants in a population of fifty.



PLATE 14

Fig. 1. *N. sylvestris* (28050P₂). Typical leaves. Scale in background ruled in dm.

Fig. 2. *N. tabacum* var. *purpurea* (28014P₆). Typical leaves.

Figs. 3-7. F₂ sesquidiploid *N. tabacum* × *sylvestris* (28264P_{2, 15, 21, 22, 7}). Extreme variants in a population of fifty.

Fig. 8. F₁ sesquidiploid *N. tabacum-sylvestris* × *sylvestris* (29287P₁). A typical leaf.

Figs. 9-10. F₁ sesquidiploid *N. tabacum-sylvestris* × *sylvestris* (29287P_{21, 12}). Extreme variants in a population of fifty.

Figs. 11-13. F₁ sesquidiploid *N. tabacum-sylvestris* × *tabacum* (29286P_{3, 20, 1}). Extreme variants in a population of fifty.

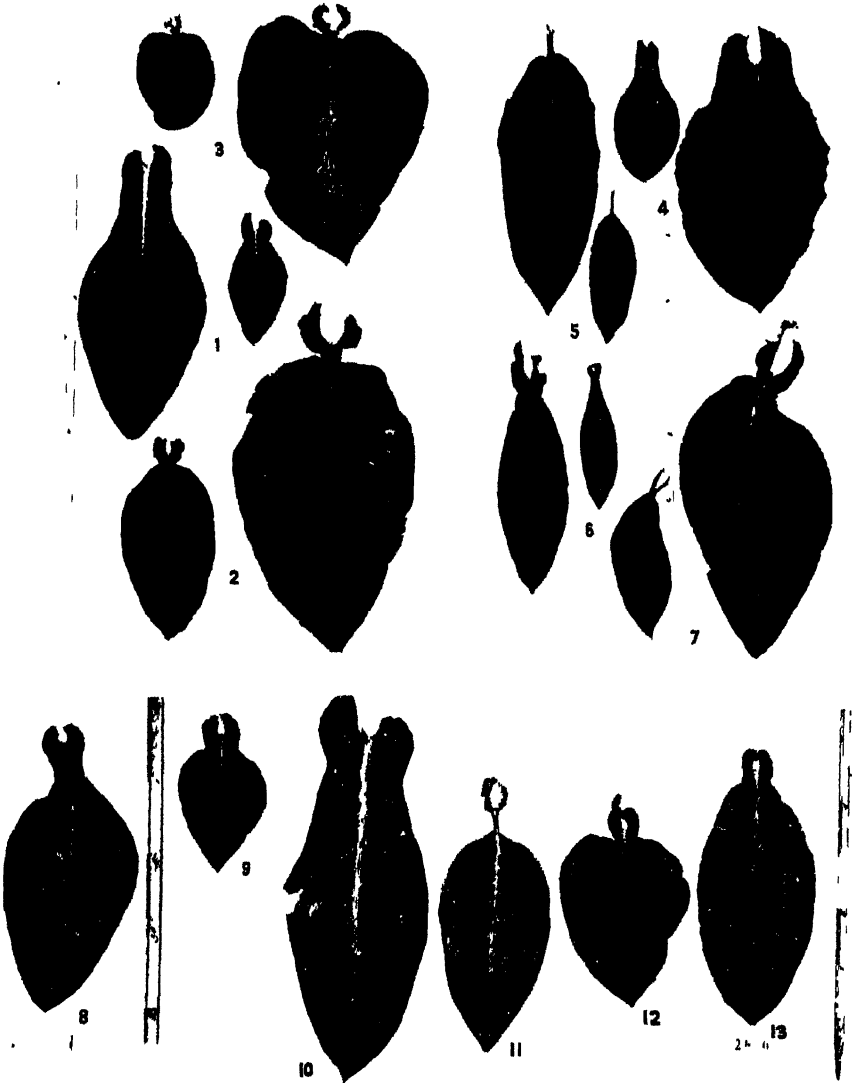


PLATE 15

Fig. 1. *N. sylvestris* (28050P₁). Typical flowers. Background ruled in cm.

Fig. 2. *N. tabacum* var. *purpurea* (28014P₈). Typical flowers.

Fig. 3. F₄ sesquidiploid *N. tabacum* × *sylvestris* (28267P₃₀). "F₃ pink type" flower of F₂ 26G209P₁₈ lineage.

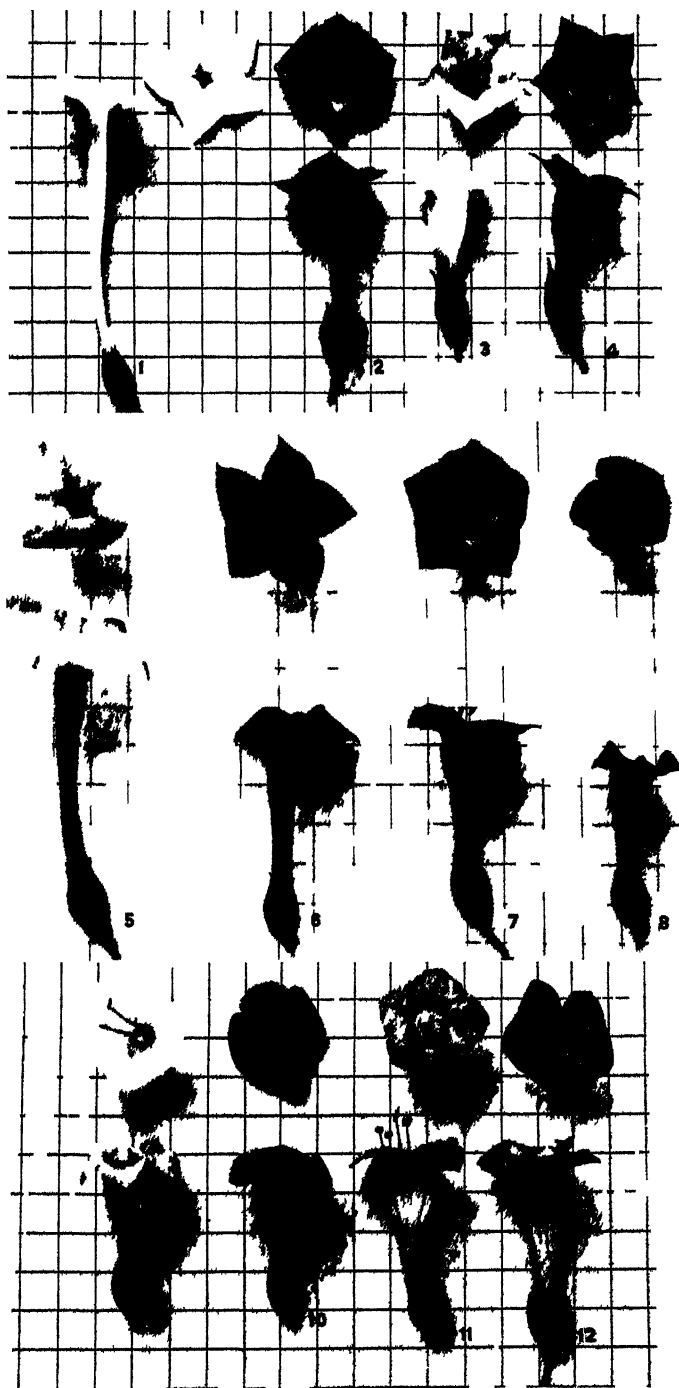
Fig. 4. F₄ sesquidiploid *N. tabacum* × *sylvestris* (28267P₄). "F₂ carmine type" flower of F₂ 26G209P₁₈ lineage.

Figs. 5-6. F₁ sesquidiploid *N. tabacum-sylvestris* × *sylvestris* (29287P_{27, 31}). Extreme variants in population of fifty.

Fig. 7. F₁ sesquidiploid *N. tabacum-sylvestris* × *tabacum* (29286P₂). A typical flower.

Fig. 8. F₁ sesquidiploid *N. tabacum-sylvestris* × *tabacum* (29286P₆). Extreme variant in a population of one hundred.

Figs. 9-12. F₂ sesquidiploid *N. tabacum* × *sylvestris* (28264P_{6, 20, 50, 18}). Extreme variants in a population of fifty.



CHROMOSOME NUMBER AND MORPHOLOGY
IN NICOTIANA

V. THE CHARACTER OF TETRAPLOID AREAS IN
CHROMOSOMAL CHIMERAS OF *N. SYLVESTRIS*,
SPEG AND COMES

BY
MILTON WEBBER

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V. THE CHARACTER OF TETRAPLOID AREAS IN CHROMOSOMAL CHIMERAS OF *N. SYLVESTRIS*, *SPEG* AND *COMES*

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In Bridges' classification (1923) of the types of quantitative chromosome alteration and chromosome reorganization, a major subdivision is concerned with the transformation of entire chromosome garnitures and the occurrence of tetraploidy affecting the entire organism or specific portions of tissue systems. Such alterations are being found characteristic of many plant species. In *Nicotiana*, haploid, triploid, and tetraploid individuals are known, and many instances of the presence of tetraploid areas in the somatic tissues of various species have been found.

On a number of occasions tetraploid cells have been seen in sections of root tips of *N. sylvestris*. The present communication consists of an analysis of the somatic chromosome morphology of this species and of a description of the nature and occurrence of tetraploid areas in its roots, which may, thus, be referred to as chromosomal chimeras.

N. sylvestris has been described and figured on a number of occasions (cf. Setchell, 1913; Goodspeed and Clausen, 1928, etc.). It is usually included in the *Petunioides* section of the genus, but is referred to by East (1928) as the "most highly specialized form of the *tabacum* section." Goodspeed and Clausen (*l. c.*) suggest that the progenitors of *sylvestris* and *tomentosa* were involved in the origin of *Tabacum*. Numerous counts in root tips and in PMC and EMC show that the chromosome number in *sylvestris* is $12n$ (pl. 16, figs. 7 and 8; text fig. 1).

In this study twenty root tips from each of six seedling *sylvestris* plants were examined. The plants were normal in character expression and gave no evidence of a diseased condition either in roots or

shoots. Roots of two of the six plants (27050P₂ and P₄) were found to be partly tetraploid. All the material was fixed in the following chrom-acetic-formalin mixture:

- 1 part, 65 c.c. water
- 10 c.c. glacial acetic acid, 1 gr. chromic acid
- 1 part 40 c.c. formalin, 35 c.c. water

Sections were stained in Heidenhain's iron haematoxylin.

Studies of chromosome number and morphology in species of *Nicotiana* are in progress in this laboratory, and the results in the case of *tabacum*, *alata*, *langsdorffii*, and *longiflora* have been presented (Ruttle, 1927, 1928; Avery, 1929; Hollingshead, 1929; Raup, MS).

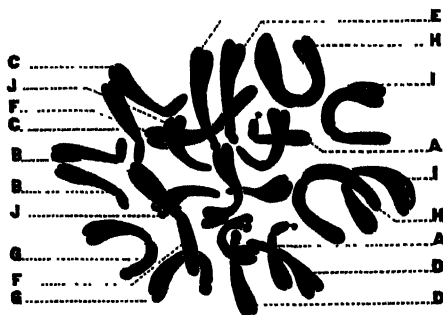


Fig. 1. *Nicotiana sylvestris*, diploid chromosome complex. The chromosomes in the center of the plate are curved and at right angles to equatorial zone. $\times 3500$.

In the case of *sylvestris*, polar views of somatic metaphases show certain characteristic morphological details which serve to distinguish this species from those previously studied here. In general, the peripheral chromosomes lie quite flat and the occurrence of satellites and the position of constrictions can be determined with considerable accuracy. The chromosomes in the center of the plate, on the other hand, stand more or less at right angles to the equatorial plane (cf. text fig. 1) and, as in *longiflora* (Hollingshead, *l. c.*), often appear curved or cut; hence they are difficult to study. Although differences in the chromosome length occur in *sylvestris*, they are not so conspicuous as in *tabacum* and *alata*. On the basis of the place of occurrence of constrictions, it is possible, however, to arrange the chromosomes in three groups. Group I (text fig. 2) possesses chromosomes with subterminal constrictions. Pair A of this group has proximal satellites, pair B is similar to A but lacks satellites, and pair C is slightly longer than either A or B and has the constriction further

from the end. Group II possesses chromosomes with approximately terminal constrictions. In this group, pair D and E, the chromosomes are similar, but those of the E pair are longer. Group III consists of chromosomes with median constrictions. In this group pair F is composed of open V's or nearly rod-shaped homologues, both arms of which are of equal length. Pair G is composed of V-shaped homologues of which one arm is slightly shorter than the other and possesses a large subspherical knob. Both arms of this pair of chromosomes are curved. Pair H is similar to G but lacks the large knob. Pair I is similar to II and G but one arm of the V is slightly shorter and is never curved. Very little can be said regarding the morphology

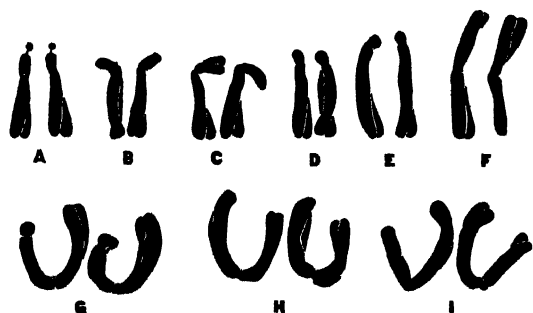


Fig. 2. *N. sylvestris*, identified pairs in the diploid chromosome complex. Pairs A, B, and C possess subterminal constrictions, pairs D and E possess approximately terminal constrictions and pairs F, G, H, and I possess median constrictions. $\times 3500$.

of the remaining three pairs of chromosomes of the somatic garniture, which lie in the center of the plate. Possibly one pair (text fig. 1, pair J) belongs to group II.

The chromosomes are often twisted in such fashion as to vary considerably in appearance. In text figure 3 evidence is given of variation in form in the case of pair A. As shown, the particular form of the two homologues is almost identical for a given metaphase plate. The particular form assumed in each case is presumably related to the stage involved; thus text figure 3*b* depicts late metaphase chromosomes while text figure 3*e* shows early metaphase chromosomes.

Often polar views gave the impression that the chromosomes are oriented in pairs, a condition illustrated in text figure 1. Generally, in such plates, the constricted portion of the chromosomes lies nearer to the center of the plate. Occasionally, however, homologues lie in opposite directions with reference to the center of the plate. In text figure 3 the center of the plate in each case is located just above the

pair. It will be seen that the homologues of *b*, *c*, *d*, and *e* bear approximately the same relation to the plate centers, while *a* homologues lie in opposite directions. Although such a condition probably does not affect the normality of mitosis, it does represent an abnormality in orientation.

Although most investigators have observed that tetraploid cells in root tips are in general larger than diploid cells, difficulties have been met with in determining the boundaries of the tetraploid sectors which result from the division of tetraploid initials. In certain cases cell size alone is not a sufficiently invariable criterion to serve as the basis on which to distinguish between $2n$ and $4n$ areas. Other cytological criteria may however be employed in this connection. Thus Lesley (1925) was able to distinguish between diploid and tetraploid cells by the size of the nuclei and of the nucleoli. However, in *syvestris* the

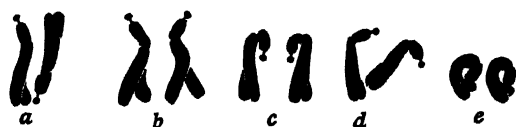


Fig. 2. *N. sylvestris*, variations in pair A. $\times 3500$.

boundaries between the two types of tissue have been rather accurately determined on the basis of cell and nuclear size. Tetraploid cells may be readily distinguished by comparing median sections in the same state of mitosis and in the same concentric row. The relative cross-section areas of $2n$ and $4n$ cells and their nuclei are indicated by the following weight relations of twenty micro-projection negative photographs:

2n weight units (gr.)		4n weight units (gr.)	
Cells	Nuclei	Cells	Nuclei
.332	.082	.591	.151

The average number of nucleoli is 2.35 for tetraploid and 1.40 for diploid cells.

Of the two plants in which $4n$ areas occurred, a single tetraploid area was found in one root of 27050P₂. It was located in the region of elongation and was composed of six epidermal cells, vertically arranged in a single row. The uppermost cell, plate 16, figure 2, was in metaphase and clearly showed the $4n$ (48) condition which is to be compared with the $2n$ (24) condition (pl. 16, fig. 1) of another epidermal cell in the same section. Considering the location of this

4n area, one naturally concludes that the initial 4n cell originated during late dermatogen cell mitoses. Its origin in a region of cell differentiation must have limited the size and extent of the sector which it produced. This sector is similar to the isolated areas of 4n cells described by Lesley (*l. c.*) in tomato.

In the other plant (27050P₄), two tetraploid chimeras were found among the twenty root tips examined. The first discovered was located in the meristematic region and was composed of a layer of periblem cells, extending in a single radial row from the plerome toward the dermatogen. It runs vertically through eleven sections, 12 μ thick, or for 132 μ . Each of the seven upper sections of the sector contain 4 tetraploid cells (pl. 16, fig. 3). The adjacent two lower sections each contain 2 tetraploid cells in the sector. These four cells are initial cells. In at least two sections of the root cap, 4n cells occur adjoining the main tetraploid sector, but since the cells in this region are very irregular in shape and arrangement, the exact number could not be determined. The occurrence of tetraploid cells in the root cap indicates that the initial 4n cell was located well down in the extreme growing point, and the lack of occurrence of 4n cells in the central cylinder suggests that it must have been located toward the periphery rather than toward the center of the root.

The second chromosomal chimera in this plant extends vertically through the growing point and region of elongation. Unfortunately the extreme apical sections of these regions were not available for study so that the extent of the chimera and the possible location of the initial 4n cell cannot be accurately determined. A careful study of this root tip was made through 35 sections of 12 μ , or for 420 μ . It occupies about one-seventh of the root area and is comparatively regular in shape. In the lowest section of the growing point (pl. 17, fig. 1) the sector is composed of three primary endodermal and eight primary cortical cells. The number of cortex cells and the shape of the sector varies somewhat in succeeding sections. Thus in section 3, one tetraploid periblem cell has failed to divide with the result that one 4n cell is isolated. Again, in section 12, an extra cell has been added to the sector, which by further division has caused the normally concentric rows of cells in the remaining sections to be irregularly disposed. The regularity in shape exhibited by the tetraploid area in all of the sections and its great extent would suggest that it has developed from an initial 4n cell situated in the growing point and, as before, toward the periphery rather than toward the center of the root. Larger

sectors similar to these in 27050P₄, have been reported by Lesley in tomato, Langlet (1927) in *Thalictrum*, Navashin (1929) and Hollingshead (1928) in *Crepis*.

In 27050P₄ tetraploid metaphases are countable in sections 3, 16, 19 and 25 (pl. 16, fig. 4; pl. 17) while in sections 7, 9, 12, 27, and 34, tetraploid cells are found which have just completed division. In the much larger diploid area, aside from the pericycle, the last indications of division are found in section 23—a metaphase. The relatively high proportion of dividing cells within the 4n area as contrasted with the infrequent metaphases observed in surrounding 2n tissue, and its topographical location indicate that 4n cells mature more slowly than normal 2n cells. This suggestion is confirmed by the fact that within the same region of the root tip diploid cells are more highly vacuolated than tetraploid. Owing to slower growth and insufficient multiplication, the descendants of an original tetraploid cell never can compete successfully with diploid cells. Unless tetraploidy successively originates within the growing point, it will probably never dominate any apical meristem. Complete tetraploid root tips, such as found by Lesley and Hollingshead, must arise from branch rootlets that have originated from a 4n sector in the pericycle.

That the larger sector in 27050P₄ is gradually reaching its maximum size is indicated by the comparatively uniform decrease in the number of tetraploid cells from the region of elongation to the growing point. Multiplication of the first formed tetraploid cells was probably fairly rapid, but on account of the faster growth and division of the normal diploid cells in the extreme growing point, these initial 4n cells are gradually being moved up into the region of elongation. If the root tip had been allowed to grow, this sector, and probably the majority of the other sectors, would have become "islands" of tetraploid cells.

There appears to be a striking contrast between tetraploidy in roots of diploid *sylvestris* and diploidy in roots of *N. tabacum* (Ruttle, 1928) and *Crepis capillaris* haplonts (Hollingshead 1928b). The diploid cells of the haplonts are by far the most vigorous cells of the entire root tip and multiplication is more rapid in the diploid cells of such a root. The descendants of a diploid root initial in a haploid plant will in all probability persist in that meristem, and possibly in time dominate the tissues of the organ. Partial diploidy in haplonts is probably considerably more frequent than tetraploidy in normal diploid plants, which may indicate that there is an inherent tendency

to the resumption of the diploid condition. The greater vigor of diploid cells in haploid plants together with the lessened vigor of $4n$ cells in diploid plants gives, perhaps, some further emphasis to this suggestion. The apparent distinctions in growth rate between haploid, diploid, and tetraploid areas make it difficult to obtain a picture, which would otherwise be presented in chromosomal chimeras, of the part played by single apical initials in the construction of the mature organ.

Polyploidy in somatic tissues will presumably be found, ultimately, to be a rather widespread phenomenon. The literature dealing with this subject has recently been summarized by Hollingshead (1928a). In *Nicotiana*, somatic polyploidy has already been found to occur in *tabacum*, *alata*, and *glutinosa* (Ruttle, Avery, l. c.; Levine, 1929). Tetraploid chimeras similar to those under consideration have recently been observed, here, in *Nicotiana Sanderae* and in a species of *Salpiglossis*.

An investigation is in progress dealing with the relative volumes of cells, nuclei, and nucleoli in haploid, diploid, triploid, and tetraploid tissues of a number of species of *Nicotiana*, to supplement the data included here as to volume relations between diploid and tetraploid areas in *sylvestris* root tips.

The writer is much indebted to Dr. T. H. Goodspeed, under whose direction the investigation was carried out, and to Dr. I. E. Webber for helpful histological advice throughout the study.

SUMMARY

1. The somatic chromosome garniture of *Nicotiana sylvestris* consists of twenty-four chromosomes. With the exception of three pairs, it is possible to analyze this somatic set on the basis of position of constrictions, and size distinctions.

2. Thus there are at least six chromosomes with subterminal constrictions, two of which have satellites, four with approximately terminal constrictions, and eight with median constrictions. The remaining six lie curved in the center of the plates in such a manner that their morphology is indistinguishable.

3. A study of tetraploid cells and areas shows that the $4n$ cells do not multiply sufficiently to compete with normal diploid cell. This condition results in isolated $4n$ areas, from which, presumably, pure tetraploid roots, and possibly shoots, may arise.

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EXPLANATION OF PLATES

PLATE 16

Plate 16, unretouched positive photomicrographs of comparable diploid and tetraploid areas, taken with Leitz "Makam" IX camera. Figures 1, 2, 3, 5, 6, 7, and 8, $\times 800$. Microscope equipped with Wratten screen No. 56, Leitz 6 objective, and 12 \times planoscopic ocular. Figure 4, $\times 500$, screen No. 56, Bausch and Lomb 4 mm. 0.65 N.A. objective and 10 \times ocular. Eastman process plates were used.

Fig. 1. *Nicotiana sylvestris* (27050P₂) root tips, diploid metaphase.

Fig. 2. Tetraploid metaphase in same section as diploid metaphase of figure 1.

Fig. 3. 27050P₂, showing the tetraploid sector in outline.

Fig. 4. 27050P₂, section 3, showing the larger tetraploid sector in outline and a 2n and a 4n metaphase.

Fig. 5. Same diploid metaphase as that shown in figure 4.

Fig. 6. Same tetraploid metaphase as that shown in figure 4.

Fig. 7. *Nicotiana sylvestris* diploid somatic metaphase from root tip, showing twenty-four chromosomes and their apparent occurrence in pairs.

Fig. 8. II-M from PMC. Twelve chromosomes in each plate.

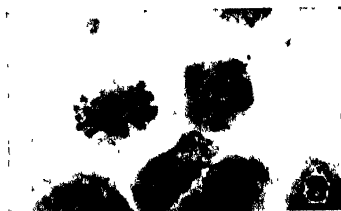
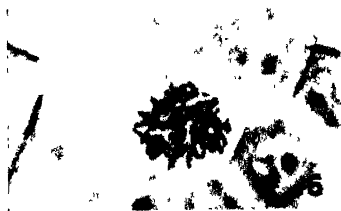
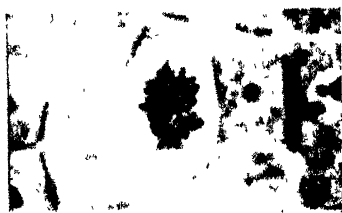
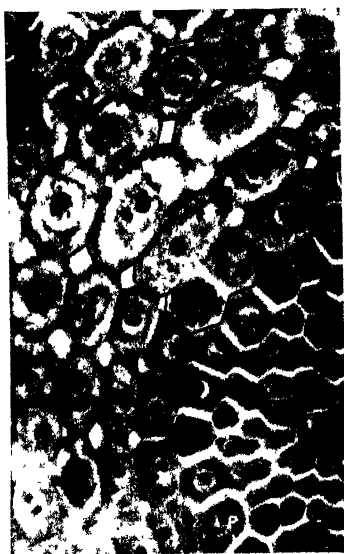
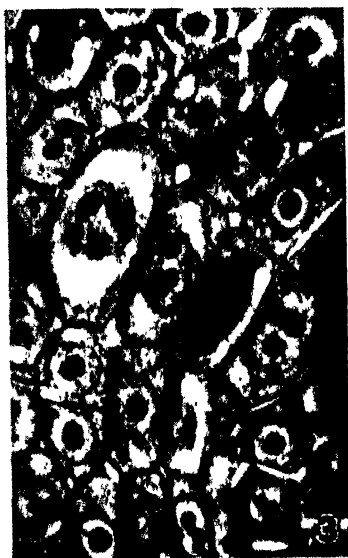
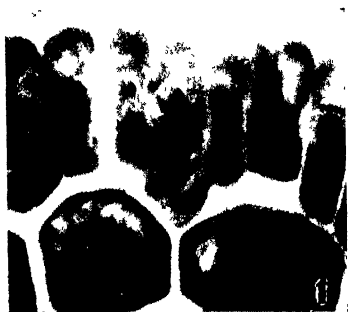
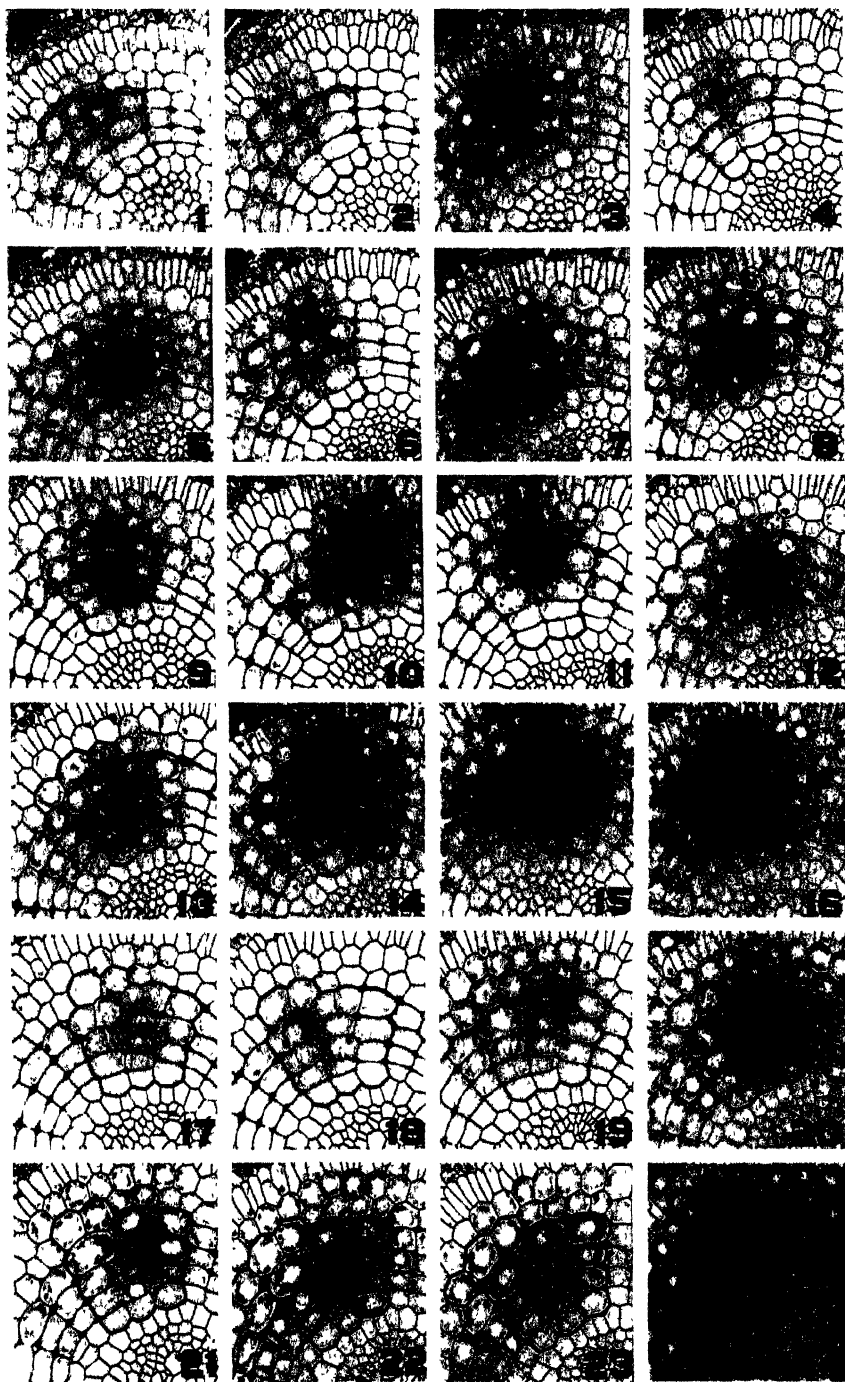


PLATE 17

Negative photomicrographs of the larger sector in 27050P₄. Cell walls drawn positive. Photographs are taken with Leitz D.R.P. micro-projection apparatus equipped with Leitz 3 objective, 12 × planoscopic ocular and Wratten screen No. 56. Eastman sensitized paper grade G No. 1 was used.

Figs. 1-24. Consecutive sections from the growing point upward, of the larger sector. Between each of the figures 9 and 10, 13 and 14, 18 and 19, 20 and 21, 21 and 22, 23 and 24, one section is left out; between figures 22 and 23 three sections are lacking.



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